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Neoadjuvant chemotherapy in breast cancer: Efficacy, response assessment and axillary strategy

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Birgitte Elisabeth Petronella Johanna Vriens

Promotor

Prof. dr. V.C.G. Tjan-Heijnen

Co-promotor

Dr. M.J.B. Aarts

Beoordelingscommissie

Prof. dr. H.J.T. Rutten (voorzitter)
Prof. dr. J.W.R. Nortier (Leids Universitair Medisch Centrum)
Dr. M.J.C. van der Sangen (Catharina Ziekenhuis Eindhoven)
Prof. dr. C.D.A. Stehouwer

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Chapter 1

General introduction and outline of the thesis

General introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide.[1] The incidence of breast cancer in the Netherlands increased the last decades to 14551 patients in 2015, with a reduction in breast cancer mortality and current 87% relative 5-year overall survival rate.[2] Survival has improved because of early detection of disease and improved treatment options. Breast cancer treated with a multidisciplinary approach involving specialist breast cancer surgical oncology, medical oncology, radiation oncology, pathology, radiology and specialist nurses has also been associated with a reduction in breast cancer mortality.[3] Systemic treatment such as chemotherapy, endocrine therapy and/or human epidermal growth factor 2 receptor targeted therapy may be indicated. Recommendations for the use of systemic therapy are based on the individual patient's risk and the balance between absolute benefit and toxicity. Overall, chemotherapy regimens based on anthracyclines and taxanes reduce breast cancer mortality by about one-third.[4,5]

Chemotherapy can be applied in the neoadjuvant or adjuvant setting. Neoadjuvant chemotherapy, also known as primary or preoperative chemotherapy, consists of chemotherapy delivered before local treatment (surgery). Neoadjuvant chemotherapy is an alternative to adjuvant chemotherapy in both early and locally advanced breast cancer and results in an equivalent disease outcome in terms of disease-free and overall survival.[6,7] Neoadjuvant chemotherapy has become the standard of care in patients with locally advanced breast cancer or borderline irresectable breast cancer. As more effective drugs have become available, interest has developed in extending this approach to patients with less advanced or resectable breast cancer. It allows observation of clinical response to systemic treatment, which may also have a positive effect on coping with the disease for the patient. For patients with earlier stages of breast cancer, down staging of the primary tumor may facilitate breast conserving therapy and bears the opportunity of down staging the axilla obviating the need of axillary treatment in some patients.[8] In a trial setting, a neoadjuvant approach is attractive as with far less patients a more rapid outcome is available in comparison to adjuvant trials.

The anthracyclines and taxanes, docetaxel and paclitaxel, represent the most potent drugs for use in breast cancer. Both the NSABP-B28 and the CALGB-9344 trials compared addition of four cycles of 3-weekly paclitaxel in sequence to four cycles of doxorubicin-cyclophosphamide chemotherapy in the adjuvant setting.[9,10] In the NSABP-B28 trial a significant improvement was seen in 5-year disease-free survival of 72% versus 76%, although without a difference in overall survival.[9] The results of the CALBG-9344 trial showed a significant difference in both disease-free and overall survival of respectively 65% versus 70% and 77% versus 80%.[10] Instead of adding taxanes after an anthracycline-containing regimen, other trials tested whether

replacement by a taxane of one or more other active cytotoxic drugs would improve outcome.[11-13] In the adjuvant PACS-01 study, replacement of the last of three cycles of 5-fluorouracil-epirubicin-cyclophosphamide by three docetaxel cycles indeed improved the 5-year disease-free survival with 5% difference (78% vs. 73%) and the overall survival with 4% (91% vs. 87%).[11] In the BCIRG-001 study, combination of docetaxel with doxorubicin and cyclophosphamide outperformed 5-fluorouracil-doxorubicin-cyclophosphamide with a 5-year disease-free survival difference of 7% (75% vs. 68%) and a 6% difference in overall survival (87% vs. 81%) in patients with node-positive breast cancer.[12] Several years later, the GEICAM 9805 trial, including patients with operable, high-risk, node-negative breast cancer, also showed an improvement in disease-free survival (88% vs. 82%) when replacing 5-fluorouracil by docetaxel.[13]

These data have accelerated the assessment of taxane-based regimens in the neoadjuvant setting. Both the Aberdeen trial and the NSAPB-B27 studied the sequential administration of docetaxel after anthracycline-based therapy versus the anthracycline regimen alone, in the neoadjuvant setting.[14,15] In the Aberdeen trial, patients with locally advanced breast cancer received four cycles of cyclophosphamide-vincristine-doxorubicin and prednisolone, and - if clinical complete or partial response was achieved - patients were subsequently randomized to receive an additional four cycles of anthracycline-based chemotherapy (cyclophosphamide-vincristine-doxorubicin-prednisolone) or to receive four cycles of 3-weekly docetaxel, whereas non-responders always received four cycles of docetaxel.[14] If an initial response to therapy was reported, docetaxel in sequence showed a significant improvement of pathologic complete response (34%) compared to patients receiving cyclophosphamide-vincristine-doxorubicin-prednisolone alone (16%).[14] In the randomized population, the use of docetaxel increased the rate of breast conserving therapy (67% vs. 48%).[16] Furthermore, docetaxel improved the 3-year overall survival (97% vs. 84%) and disease-free survival (90% vs. 77%) as compared to those receiving eight cycles of cyclophosphamide-vincristine-doxorubicin-prednisolone.[16] The large NSABP-B27 trial randomized patients with stage I-III breast cancer to one of three treatment groups: four cycles of neoadjuvant doxorubicin-cyclophosphamide, four cycles of neoadjuvant doxorubicin-cyclophosphamide followed by four cycles of docetaxel, or four cycles of neoadjuvant doxorubicin-cyclophosphamide followed by surgery and four cycles of adjuvant docetaxel.[15] Again, the addition of neoadjuvant docetaxel significant increased the pathologic complete response rate (26% vs. 14% with anthracyclines alone).[15] Patients with a complete pathological response (in all treatment arms) had a superior survival as compared to those that did not reach complete pathological response.[17] However, despite clear improvement of pathologic complete response, addition of a taxane did not impact the disease-free or overall survival when compared with patients treated with only four cycles of doxorubicin-cyclophosphamide. Evans *et al*. studied substitution of cyclophosphamide for

docetaxel, thereby comparing 6 cycles of neoadjuvant doxorubicin-docetaxel with doxorubicin-cyclophosphamide.[18] No difference was seen in pathologic complete response rate, and no difference was seen in relapse rate, distant metastases and overall survival between the two treatment arms after a median follow-up of 32 months.[18] In this study, the exchange of one active drug by another did not lead to an improved outcome.

In general, taxane-containing chemotherapy improved efficacy and it is accepted worldwide that taxanes should be incorporated in the (neo)adjuvant treatment of breast cancer patients at increased risk of relapse. Moreover, the use of anthracyclines and alkylating agents such as cyclophosphamide appear to be important. The most optimal strategy for incorporating docetaxel to the use of these other agents is, however, still under investigation.

Aims and outline of the thesis

Both, the upfront combination of docetaxel with anthracyclines and cyclophosphamide and the sequential use of docetaxel after anthracyclines and cyclophosphamide have shown to improve breast cancer outcome. We considered it relevant to investigate the most optimal strategy for incorporating docetaxel in the neoadjuvant setting.

Although in general taxanes did improve survival in early breast cancer trials, they did not do so in metastatic breast cancer trials. For that reason, we decided first to systematically review taxane-based chemotherapy studies in early and metastatic breast cancer **in chapter 2**, to assess if factors such as study design or comparator may have caused the different outcome on efficacy endpoints.

Based on prior performed studies, we hypothesized that the planned chemotherapy dose and dose-intensity might be critical factors for the outcome with the assumption, that delivering chemotherapy within a shorter time frame could prevent tumor outgrowth and development of resistance and might thus be more efficacious than sequential regimens in which the chemotherapy is given in a larger time frame.[19] We also argued, that the combined docetaxel-doxorubicin-cyclophosphamide regimen, with a higher total dose delivered in a shorter time period (that is a higher dose-intensity), might be more effective than doxorubicin-cyclophosphamide followed by docetaxel, although we acknowledged that for the triplet the dose-intensity per drug per cycle was actually lower than for the sequential regimen.

Chapter 3 describes the randomized, national, multicenter phase III INTENS study which was designed to determine whether delivering taxane-containing neoadjuvant

chemotherapy in a shorter period of time improved outcome in breast cancer patients. Women with newly diagnosed breast cancer were eligible if they had a primary tumor size of 3 cm or more and/or presence of positive regional lymph nodes. They were randomly assigned to neoadjuvant chemotherapy of four cycles of doxorubicin and cyclophosphamide (60/600 mg/m^2) followed by four cycles of docetaxel (100 mg/m^2) or six cycles of docetaxel-doxorubicin-cyclophosphamide (75-50-500 mg/m^2) every three weeks. The primary endpoint was the pathologic complete response rate, defined as no invasive tumor present in the breast. In **chapter 4**, we report the 5-year disease-free survival and overall survival data.

A combination of anthracycline, cyclophosphamide and a taxane is very myelotoxic with a substantial risk of febrile neutropenia.[20,21] Prophylaxis with granulocyte-colony stimulating factor (G-CSF) reduces the severity and duration of chemotherapy induced neutropenia. According to international guidelines, primary G-CSF prophylaxis is indicated in case the risk of febrile neutropenia is more than 20%, which may be due to the chemotherapy schedule and/or due to specific patient factors such as higher age or lower performance status.[22] The triplet docetaxel-doxorubicin-cyclophosphamide schedule, as used in the aforementioned INTENS study can be considered as a schedule with increased risk of febrile neutropenia. In the Dutch Two-to-Six trial, breast cancer patients treated with 3-weekly chemotherapy with increased risk of febrile neutropenia were randomly assigned to primary G-CSF prophylaxis during all chemotherapy cycles or primary G-CSF prophylaxis limited to the first two chemotherapy cycles only.[23] Most of included patients were treated with the triplet docetaxel-doxorubicin-cyclophosphamide schedule. The Two-to-Six study was designed, based on the observation that febrile neutropenia is mostly seen during the first two chemotherapy cycles. Limiting G-CSF prophylaxis to the first cycles might be a more cost-effective strategy, so we hypothesized. However, the study showed that limiting primary G-CSF prophylaxis was not effective, with febrile neutropenia occurring in 10% of patients treated with G-CSF prophylaxis during all cycles compared to 36% of patients treated with G-CSF prophylaxis only in the first two chemotherapy cycles, with a peak incidence in the third chemotherapy cycle (24% of patients).[23] In **chapter 5** we evaluated more in detail the chemotherapy-induced hematologic toxicity for the triplet docetaxel-doxorubicin-cyclophosphamide schedule, based on data of the Dutch phase III Two-to-Six study. We assessed if there was a protective effect of prior chemotherapy or prior use of granulocyte-colony stimulating factor on the blood cell counts in the next chemotherapy cycle. For this analysis, patients who developed FN during treatment, or who underwent modifications of chemotherapy or G-CSF treatment during treatment were excluded. The primary endpoint was the nadir blood cell counts over cycles 1 through 6 for patients in the standard arm and over cycles 3 through 6 for patients in the experimental arm.

In the neoadjuvant setting, conflicting findings are reported about accuracy of magnetic resonance imaging and ultrasound for assessment of chemotherapy responsiveness. Some studies suggest that breast magnetic resonance imaging is more reliable than any of the conventional methods in the assessment of residual tumor tissue, while other studies suggest that there is no difference between magnetic resonance imaging and ultrasound.[24,25] In **chapter 6** we compared the accuracy of clinical breast tumor size measurement post neoadjuvant chemotherapy by magnetic resonance imaging and ultrasound with the post-neoadjuvant pathologic tumor size as gold standard.

Neoadjuvant chemotherapy has shown to eradicate nodal disease in 20% to 40% of the patients.[26] Performing a sentinel node biopsy post-neoadjuvant chemotherapy might be an attractive strategy to take maximum benefit of its effect on nodal down staging and may potentiate axilla-conserving treatment. In **chapter 7** we reviewed the literature regarding the accuracy of sentinel biopsy post-neoadjuvant chemotherapy in patients with clinically node-negative and node-positive disease before start of neoadjuvant chemotherapy. Obviously, if the axillary lymph nodes are truly negative, there can be no possible benefit from performing an axillary lymph node dissection. For this reason we investigated in **chapter 8** the impact of timing of axillary staging pre- versus post- neoadjuvant chemotherapy on final pathologic node-negative rate in patients with clinically node-negative breast cancer. Secondary endpoint was the usability of the sentinel node procedure in patients with clinically node-positive disease that converted to cN0 after neoadjuvant chemotherapy. Patients were selected from two sequentially conducted Dutch phase III trials, the INTENS trial (presented in chapters 3 and 4) and the NEO-ZOTAC trial (studying the impact of two neoadjuvant chemotherapy schedules and use of zoledronic acid on complete pathologic response rate).[27] For the present analysis, patients were excluded if they had not undergone surgical axillary staging.

Finally, in **chapter 9** we present a summary, the general conclusions and future perspectives.

To conclude, specific research questions in the thesis are:

1. Are the chosen comparator and/or study design in taxane-containing chemotherapy regimens in metastatic and early breast cancer trials of influence on efficacy endpoints? **Chapter 2**
2. Is there a difference in the pathologic complete response rate in the breast in patients with newly diagnosed non-metastatic breast cancer when comparing two different taxane-containing neoadjuvant chemotherapy regimens? **Chapter 3**
3. Is there a difference in 5-year disease-free and overall survival in patients with newly diagnosed non-metastatic breast cancer when comparing two different taxane-containing neoadjuvant chemotherapy regimens? **Chapter 4**

4. Is there a protective effect of prior anthracycline-taxane-containing chemotherapy with or without prior granulocyte-colony stimulating factor on the next cycle blood cell counts? **Chapter 5**
5. Is there a difference in accuracy of clinical breast tumor size measurement post-neoadjuvant chemotherapy by magnetic resonance imaging versus ultrasound? **Chapter 6**
6. What is the accuracy of sentinel node biopsy post-neoadjuvant chemotherapy? **Chapter 7**
7. What is the impact of timing of axillary staging pre- versus post-neoadjuvant chemotherapy on final pathologic node-negative rate in patients with clinically node-negative breast cancer? **Chapter 8**

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Chapter 2

If there is no overall survival benefit in metastatic breast cancer: does it imply lack of efficacy? Taxanes as an example

Birgit EPJ Vriens, Dorien JA Lobbezoo, Joep PJ de Hoon, Jürgen Veeck,
Adri C Voogd, Vivianne CG Tjan-Heijnen

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Abstract

In recent years, new drugs have shown activity in metastatic breast cancer, but not always resulting in an overall survival benefit. This has led to discussions if such drugs, mainly expensive drugs, should be reimbursed especially when also not leading to improvement in quality of life. For that reason, we decided to systematically review taxane-based chemotherapy studies in early and metastatic breast cancer, to assess which factors may have caused the differential outcome. Taxanes did not improve survival in metastatic breast cancer trials, whereas they did so in early breast cancer trials. We questioned if the differential outcome of taxanes in metastatic breast cancer might be caused by the chosen comparator and study design. We noticed that in the majority of metastatic breast cancer studies taxanes were used as a substitute for other active cytotoxic drugs, mainly cyclophosphamide, whereas in early breast cancer studies taxanes were generally delivered in addition to a standard regimen. We conclude from our analyses that use of taxanes instead of other active drugs explains the lack of overall survival benefit in metastatic breast cancer trials. Further, our results suggest that cyclophosphamide is an important drug in the treatment of breast cancer, being as effective as optimally dosed taxanes and anthracyclines. By studying the different study designs and comparators in both settings, we were able to demonstrate their impact on efficacy endpoints. We conclude, therefore, that re-assessment of studies of drugs both assessed in metastatic and early breast cancer provides a new tool for improved understanding.

Introduction

Taxanes are approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for use in metastatic and early breast cancer. Despite registration, it was concluded in the most recent Cochrane review that there was no evidence of a survival benefit in metastatic breast cancer for single-agent taxanes versus anthracyclines.[1] And, although an overall survival benefit was seen in favour of taxane containing regimens, the superiority of taxane regimens over non-taxane containing regimens was not seen if the non-taxane regimens were largely limited to optimally-dosed anthracycline-based regimens.[1] In a second review on taxanes in metastatic breast cancer, it was concluded that single-agent taxane was even less effective than single-agent anthracycline, based on the EORTC study 10923.[2] On the other hand, it was concluded that first-line anthracycline-taxane combinations seemed slightly better than anthracycline-based regimens with regard to tumour response and progression-free survival, although not with regard to overall survival. However, the conclusions from meta-analyses on adjuvant taxane breast cancer trials were remarkably different, showing an improvement in both disease-free and overall survival.[3-5]

In recent years, new drugs have shown activity in metastatic breast cancer, but not always resulting in an overall survival benefit.[6] In the oncology community this has led to discussions on whether such drugs should be reimbursed. A thorough analysis of drugs without a clear survival gain in metastatic breast cancer but with a survival gain in early breast cancer may help clarify this apparent discrepancy, and may prevent premature conclusions on the value of new drugs in this field. For that reason, we decided to systematically review taxane-based chemotherapy studies in early and metastatic breast cancer, to assess which factors may have caused the different outcomes between these patient groups.

Methods

Search strategy and selection criteria

A detailed search strategy, consisting of numerous MeSH heading and text word combinations, “breast cancer”, “chemotherapy”, “taxanes”, “docetaxel” and “paclitaxel”, was used to search the PubMed database. Publications of clinical phase III trials between 1995 and November 1, 2010 in the English language were included. Abstracts of the annual meetings of the American Society of Clinical Oncology (ASCO) and of the San Antonio Breast Cancer Symposium (SABCS) were searched for relevant trials (and replaced by full papers if published before November 1, 2011).
Trials with the taxanes docetaxel and paclitaxel in combination with or compared to anthracyclines, and used within its licensed indications, as well as comparator regimens which are considered as standard of care, were eligible. Consequently, we

excluded trials that used first generation chemotherapy schemes, other combinations with no licensed indication, as well as trials including pegylated doxorubicin or nab-paclitaxel or comparing different taxanes.

Study categories and endpoints

Our primary research question was whether we could identify factors that contributed to the differential outcome of taxanes in metastatic versus early breast cancer setting. For that, we categorised the studies by disease setting, by choice of taxane and by study design, i.e., substitution of another active drug or addition of a taxane without substitution. Finally, we addressed the most optimal use of taxanes, sequential or concurrent use. Studies in which both arms either used concurrent or sequential taxanes were excluded.[7]

Data and statistical methods

Data on the outcomes of interest were: response rate and progression-free survival in metastatic breast cancer, disease-free survival in early breast cancer and overall survival in both settings. Because of immature follow up we mainly report on disease-free survival in early breast cancer studies. Trials were not consistent in the way they defined progression-free or disease-free survival; out of convenience we analysed all these as if they reported on progression-free and disease-free survival in a similar way. If odds ratios or hazard ratios were not provided, they were obtained using the available summary statistic.[8] If insufficient data were available the trials were not included in the pooled analysis.

We performed a pooled analysis using the Review Manager software (RevMan 5) provided by the Cochrane Collaboration. We used the fixed or random effect model, based on observed minus expected number of events and the variance of each trial. Chi-square tests were used to test for heterogeneity over all trials included in the pooled analyses. Due to the limited number of trials in each pooled analysis, no sensitivity analysis could be performed when significant heterogeneity occurred among the trials.

Results

Overall efficacy of taxanes in metastatic and early breast cancer

In total 10 trials in the metastatic breast cancer setting were included, comparing taxane containing chemotherapy schemes with anthracycline containing schemes (Table 2.1). We calculated the pooled hazard ratio and found no significant difference for progression-free survival with a hazard ratio of 0.94 (95% CI 0.88 to 1.01) and for overall survival with a hazard ratio of 0.98 (95% CI 0.91 to 1.05) (Figure S2.A, Appendix 2).

Table 2.1 Treatment effects of anthracycline-taxane combinations in metastatic breast cancer in first line.

Author	Study	Comparison	N	Response rate (%)				Median PFS (months)				Median OS (months)			
				Exp	Con	OR	95%CI	Exp	Con	HR	95% CI	Exp	Con	HR	95% CI
		Substitution													
		T vs A													
Chan[11]	Tax 303	D 100 vs A 75	326	48	33	1·80	1·10 - 2·90	6·0	4·8	1·00	0·80 - 1·30	15·0	14·0	1·00	0·80 - 1·30
Paridaens[12]	EORTC 10923	P 200 vs A 75	331	25	41	0·50	0·31-0·81	3·9	7·5	1·69	1·33-2·13	15·6	18·3	1·16	0·90-1·49
Sledge[10]	ECOG E1193	P 175 vs A 60	453	34	36	0·91	0·62-1·34	6·3	6·0	0·95	0·77-1·16	22·5	19·1	0·95	0·77-1·16
		AT vs AC or ET vs EC													
Nabholtz[13]	Tax 306	AD 50/75 vs AC 60/600	429	59	47	1·68	1·15 - 2·46	8·6	7·4	0·76	0·60 – 0·94	22·5	21·7	0·89	0·72 - 1·09
Blohmer[14]	NOGGO EV Germany	ED 75/75 vs EC 90/600	240	47	42	1·22	0·73 - 2·04	10·3	10·1	0·98	P = 0·38	30·0	19·9	0·66	P = 0·21
Langley[15]	UK NCR I ABO1	EP 75/200 vs EC 75/600	705	65	55	1·49	1·11 - 2·02	7·0	7·1	1·07	0·92 - 1·24	13·0	14·0	1·02	0·87 - 1·19
Lück#[16]	AGO	EP 60/175 vs EC 60/600	516	51	39	1·64	1·16 - 2·33	9·2	7·4	0·94	0·78 - 1·13	19·1	22·1	1·16	0·94 - 1·43
Biganzoli[17]	EORTC 10961	AP 60/175 vs AC 60/600	275	58	54	1·17	0·73 - 1·89	6·0	6·0	0·94	0·74 - 1·20	20·6	20·5	1·11	0·83 - 1·49
		AT vs FAC													
Bontenbal[18]	-	AD 50/75 vs FAC 500/50/500	216	58	37	2·29	1·33 - 3·96	8·0	6·6	0·67	0·51 - 0·88	22·6	16·2	0·70	0·52 - 0·94
Jassem[19]	CCEI Pac BCSG	AP 50/220 vs FAC 500/50/500	267	66	53	1·64	0·99 - 2·70	8·1	6·2	0·77	0·58 – 1·01	23·3	18·3	0·69	0·51 - 0·93
		Addition													
		AT vs A													
Sledge[10]	ECOG E1193	AP 50/150 vsA60	454	47	36	1·56	1·07 - 2·28	8·2	6·0	0·78	0·63 - 0·96	22·4	19·1	1·00	0·81 - 1·20
		Sequential vs concurrent													
		E-T or A-T vs ET or AT													
Conte[42]		E120-P250 vs EP 90/200	198	58	59		NS	10·8	11		NS	26	20		NS
Alba[20]	GEICAM 9903	A75-D100 vs AD 50/75	144	61	51	1·54	0·79 - 2·99	10·5	9·2	0·97	0·68 - 1·39	22·3	21·8	1·24	0·74 - 2·06

A =adriamycin, C=cyclophosphamide, CI=confidence interval, Con=control arm, D=docetaxel, E=epirubicin, Exp=experimental arm, F=5-fluorouracil, FU=follow-up, HR=hazard ratio, N=number of patients, NS=non significant, OR=odds ratio, OS=overall survival, P=paclitaxel, PFS=progression- free survival, T=taxane, #=abstract; hazard ratios abstracted from the review of Piccart et al.[2]

In total 21 trials in the adjuvant breast cancer setting were included, comparing chemotherapy schemes with and without taxanes (Table 2.2). We were able to calculate pooled hazard ratios for 14 trials, with regard to overall survival. The pooled analysis showed a hazard ratio for disease-free survival of 0.85 (95% CI 0.80 to 0.91) in favour of adding a taxane. Moreover, a significant difference in favour of taxanes was seen in overall survival (hazard ratio 0.85; 95% CI 0.79 to 0.91) (Figure S2.B, Appendix 2).

Efficacy according to the choice of taxane in metastatic and early breast cancer

In our pooled analysis of metastatic breast cancer studies, there was a trend for improved overall survival with a hazard ratio of 0.88 (95% CI 0.76 to 1.01) when only including studies using docetaxel (Figure S2.A, Appendix 2). In contrast, overall survival was not improved in metastatic breast cancer studies using paclitaxel with a hazard ratio of 1.01 (95% CI 0.93 to 1.10) (Figure S2.A, Appendix 2).
On the other hand, in early breast cancer docetaxel and paclitaxel resulted in similar improvements in overall survival both with a hazard ratio of 0.85 (95% CI 0.77 to 0.94) (Figure S2.B, Appendix 2). In all but one study on paclitaxel a 3-weekly schedule was used.[9]

Substitution or addition of taxanes in metastatic breast cancer

In metastatic breast cancer most studies investigated substitution, either by use of taxanes as single-agent versus single-agent anthracyclines or by use of taxanes in combination with anthracyclines versus anthracycline-cyclophosphamide combinations.

Substitution: taxanes versus anthracyclines as single–agent

The registration of single-agent docetaxel was based on the TAX 303 trial. Patients received either 3-weekly docetaxel 100 mg/m² or adriamycin 75 mg/m² (Table 2.1).[11] With respect to response rate docetaxel was superior. Progression-free survival and overall survival were not improved. There are two studies comparing paclitaxel with anthracyclines (Table 2.1).[10,12] In the EORTC 10923 trial, patients were randomised to receive either 3-weekly paclitaxel 200 mg/m² or adriamycin 75 mg/m².[12] In the North-American ECOG E1193 trial patients were randomised to receive single-agent paclitaxel (3-weekly 175 mg/m²) or single-agent adriamycin (3-weekly 60 mg/m²).[10] Compared with a relative low dose adriamycin, 3-weekly paclitaxel showed similar efficacy.
Our pooled analysis shows a hazard ratio for overall survival of 1.02 (95% CI 0.89 to 1.16), showing that single-agent taxanes are equally effective as single-agent anthracyclines in first and second line treatment of metastatic breast cancer (data not further shown). It is noted that the taxane choice and anthracycline dose both may have influenced the individual study outcomes.

Table 2.2 Treatment effects of anthracycline-taxane combinations in early breast cancer.

Author	Study	Comparison	N	No of cycles		5-years DFS				5-years OS			
				Exp	Con	%Exp	%Con	HR	95% CI	%Exp	%Con	HR	95% CI
		Substitution											
		TC vs AC											
Jones[21, 29]	US Onc 9735	DC 75/600 vs AC 60/600	1016	4	4	86	80	0·67	0·50-0·94	90	87	0·76	0·52-1·1
		TAC vs FAC											
Martin[22]	BCIRG 001	DAC 75/50/500 vs FAC 500/50/500	1491	6	6	75	68	0·72	0·59-0·88	87	81	0·70	0·53-0·91
Martin[23]	GEICAM 9805	DAC 75/50/500 vs FAC 500/50/500	1060	6	6	88	82	0·68	0·49-0·93	95	94	0·76	0·45-1·26
		AT vs AC											
Goldstein[24]	ECOG E2197	AD 60/60 vs AC 60/600	2882	4	4	85	85	0·98	0·82-1·16	92	91	0·94	0·76-1·18
Brain#[25]	RAPP-01	AD 50/75 vs AC 60/600	627	4	4	91	91	0·91	0·54-1·52				
Francis[26]	BIG 2-98	AD-CMF 50/75 vs AC 60/600-CMF	1446	4+3	4+3	74	72	0·93	0·75-1·14				
		ET vs FEC											
Roche#[27]	PACS04	ED 75/75 vs FEC 500/100/500	3010	6	6	82	80	0·89	0·76-1·05	90	90	1·07	0·85-1·35
Mastro#[28]	Gono MIG5	EP 90/175 vs FEC 600/60/600	1055	4	6	70	71	NS		88	89	NS	
		Taxane in sequence (part. substitution)											
		T-EC or EC-T vs FEC											
Polyzos[30]	T-EC vs FEC	D100-EC 75/700 vs FEC 700/75/700	756	4+4	6	73	67	0·77	0·59 - 0·99	84	81	0·91	0·66-1·26
Nitz#[31]	EC-Doc	EC 90/600-D100 vs FEC 500/100/500	1837	4+4	6	90	86	0·67	0·48-0·90	95	93	0·63	0·40-0·98
		FEC-T vs FEC or T-FAC vs FAC											
Ellis[32]	UK-TACT	FEC-D100 vs FEC 600/60/600 or E-CMF	4162	4+4	8	76	74	0·95	0·85-1·08				
Roché[33]	PACS 01	FEC-D100 vs FEC 500/100/500	1999	3+3	6	78	73	0·82	0·69-0·99	91	87	0·73	0·56-0·94
Martin[9]	GEICAM 9906	FEC-P100 vs FEC 600/90/600	1248	4+8wkl	6	79	72	0·77	0·62-0·95	90	87	0·78	0·57-1·06
Buzdar[34]	MDACC	P250-FAC vs FAC1000/50/500	524	4+4	8	86	83	0·70	0·47-1·06				
		EC-T or AC-T vs Can CEF($Q4_w$)											
Jannì#[35]	ADEBAR study	EC 90/600-D100 vs Canadian CEF_{120}	1502	4+4	6			1·14	0·89-1·45			1·01	0·74-1·37
Burnell[36]	MA21	AC60/600-P175 vs Canadian CEF_{120}	1403	4+4	6	85	90	1·49	1·12-1·99				
Burnell[36]	MA21	EC120/830Q2wk-P175 vs Canadian CEF_{120}	1402	6+4	6	90	90	0·89	0·64-1·22				

Table 2.2 (continued)

Author	Study	Comparison	N	No of cycles		5-years DFS				5-years OS			
				Exp	Con	%Exp	%Con	HR	95% CI	%Exp	%Con	HR	95% CI
		Addition											
		AT vs A											
Gianni[37]	ECTO	AP60/200-CMF vs A75-CMF	904	4+4	4+4	76	69	0·73	0·57-0·97	85	85	0·80	0·56-1·07
		A(C)-T vs A(C) or E-T vs E											
Francis[26]	BIG 2-98	A75-D100-CMF vs A75-CMF	1441	3+3+3	4+3	78	73	0·79	0·64-0·98				
Bianco#[38]	TAXit- 216	E 120-D100-CMF vs E120–CMF	972	4+4+4	4+4	74	67	0·78	0·61-1·00			0·74	0·51-1·07
Mamounas[39]	NSABP-B28	AC60/600-P225 vs AC60/600	3060	4+4	4	76	72	0·83	0·72-0·95	85	85	0·93	0·78-1·12
Henderson[40]	CALGB 9344	$AC_{(60,75,90)}$/600-P175 vs $AC_{(60,75,90)}$/600	3121	4+4	4	70	65	0·83	0·73-0·94	80	77	0·82	0·71-0·95
Fountzilas[41]	HeCOG	E110-P250-CMF vs E110-CMF	595	3+3+3	4+4	70	68	0·93	0·69-1·24	80	77		
		Sequential vs concurrent											
		A(C)-T vs AT or TAC											
Francis[26]	BIG 2-98 A-D vs AD	A75-D100-CMF vs AD 50/75-CMF	1919	3+3+3	4+3	78	74	0·83	0·69-1·00				
Swain[43]	NSABP-B30 AC-D vs AD	AC60/600-D100 vs AD 50/75	3506	4+4	4	74	69	0·80	0·70-0·91	83	79	0·83	0·70-0·98
Swain[43]	NSABP-B30 AC-D vs DAC	AC60/600-D100 vs DAC 75/50/500	3511	4+4	4	74	69	0·83	0·73-0·95	83	79	0·86	0·72-1·02
Eiermann[44]	BCIRG 005 AC-D vs DAC	AC 60/600-D100 vs DAC 75/50/500	3298	4+4	6	79	79	1·00	0·86-1·16				

A=adriamycin, C=cyclophosphamide, CI=confidential interval, Con=control arm, D=docetaxel, DFS= disease-free survival, E=epirubicin, Exp=experimental arm, F=5-fluorouracil, HR=hazard ratio, M=methotrexate, N=number of patients, NS=non significant, OS=overall survival, P=paclitaxel, T=taxane, #=abstract.

Substitution: anthracycline-taxane combinations versus AC or FAC

There are five phase III trials that report on the comparison between an anthracycline-taxane combination regimen (AT) and an anthracycline-cyclophosphamide (AC) combination regimen, two of them using docetaxel and three of them using paclitaxel as taxane (Table 2.1).[13-17] The TAX 306 study led to the registration of the AT-regimen for the treatment of metastatic breast cancer.[13] Patients received either 3-weekly AT (adriamycin 50 mg/m² and docetaxel 75 mg/m²) or AC (adriamycin 60 mg/m² and cyclophosphamide 600 mg/m²). Compared with AC, the AT-regimen proved to be superior in terms of response rate and progression-free survival, but equal in terms of overall survival. The other, smaller, trial using docetaxel showed no difference in efficacy.[14] The three trials using paclitaxel showed comparable progression and overall survival for AT when compared with AC, noting that the comparator regimen used a lower than standard anthracycline dose in two of three studies.[16,17]

There are two phase III trials that report on the comparison between anthracycline-taxane doublet regimen (AT) and the 5-fluorouracil-anthracycline-cyclophosphamide (FAC) triplet regimen, one using docetaxel and one paclitaxel as taxane (Table 2.1).[18,19] The first trial of Bontenbal *et al.* randomised 216 patients to receive either AT (adriamycin 50 mg/m^2 and docetaxel 75 mg/m²) or FAC (500/50/500 mg/m²).[18] Due to slow accrual, the trial was stopped prematurely. Even so, AT was superior with respect to all efficacy endpoints. This result was confirmed in a multicentre trial of 267 patients, receiving either the same schedule of FAC or adriamycin 50 mg/m² in combination with 3-weekly paclitaxel 220 mg/m².[19] At a median follow-up of 69 months, compared with FAC, the AT regimen proved to be superior in terms of response rate, progression-free and overall survival.

In our pooled analysis combining the 6 trials with available survival data in which an anthracycline-taxane combination is compared with an anthracycline containing regimen without a taxane, overall survival was not significantly different (hazard ratio of 0.92; 95% CI 0.78 to 1.09) but results showed a statistically significant heterogeneity (Figure 2.1). When analysing the studies with different comparators separately, as discussed above, this heterogeneity disappeared. AT appeared to be as effective as AC (hazard ratio of overall survival 1.03; 95% CI 0.92 to 1.15), whereas AT was superior compared to the triplet FAC (hazard ratio of overall survival 0.69; 95% CI 0.56 to 0.85) (Figure 2.1). Apparently, study outcome is affected by the chosen comparator.

Addition: concurrent use of taxanes with an anthracycline

Only one study assessed this concept in metastatic breast cancer (Table 2.1).[10] Patients were either treated with single agent 3-weekly adriamycin (60 mg/m²), single-agent 3-weekly paclitaxel (175 mg/m^2) or with the combination of 3-weekly paclitaxel (150 mg/m²) and adriamycin (50 mg/m²).[10] Compared with single-agent anthracycline the combination treatment was superior in terms of response rate (odds ratio 1.56; 95% CI 1.07 to 2.28) and progression-free survival (hazard ratio 0.78;

95% CI 0.63 to 0.96), but not in terms of overall survival. Of note, 57% of patients in the single-agent arm crossed over to the other arm with the combination treatment.

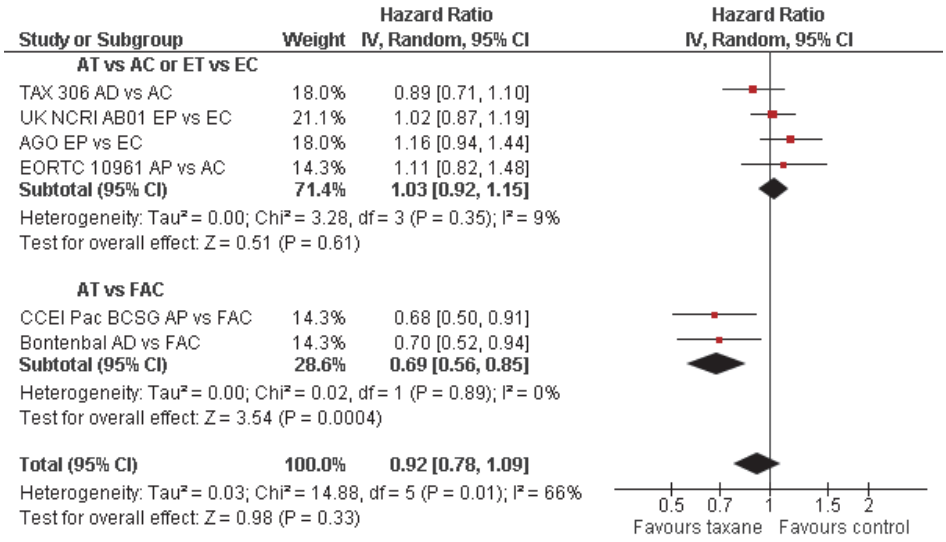

Figure 2.1 Taxane (+/- anthracycline) regimens versus anthracycline regimens in metastatic breast cancer, hazard ratio of overall survival. Subgroups are based by the way the taxanes are used. A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, P=paclitaxel, T=taxane.

Substitution or addition of taxanes in early breast cancer

In the adjuvant setting taxanes were studied either by substituting one or more other cytotoxic drugs, or added concurrently or in sequence to anthracyclines.

Substitution: taxanes instead of anthracyclines, cyclophosphamide and/or 5-fluorouracil

Of the eight studies that assessed the impact of substitution, only one study used paclitaxel, in a 3-weekly schedule (Table 2.2).[20-28] In a US Oncology study, patients were either treated with four cycles of docetaxel (75 mg/m²) combined with cyclophosphamide (600 mg/m²; TC) or four cycles of adriamycin (60 mg/m²) and cyclophosphamide (600 mg/m²; AC).[29] The taxane containing regimen resulted in a significant improvement of disease-free survival and overall survival.[21] In two studies docetaxel (75 mg/m²) was given instead of 5-fluorouracil (500 mg/m²), in combination with adriamycin (50 mg/m²) and cyclophosphamide (500 mg/m²), that is TAC versus FAC.[22,23] The disease-free and overall survival in both studies were significantly better in the taxane containing arm.

Cyclophosphamide was replaced by docetaxel in three studies, in combination with adriamycin: AT versus AC.[24-26] None of the trials showed superiority for the docetaxel-adriamycin combination regimen.

Two studies compared a concurrent regimen of anthracyclines and taxane (ET) with FEC, one using docetaxel and one paclitaxel.[27,28] The PACS 04 study randomised to receive either ED (epirubicin 75 mg/m^2 and docetaxel 75 mg/m²) or FEC (5-fluorouracil 500 mg/m², epirubicin 100 mg/m² and cyclophosphamide 500 mg/m²).[27] The other trial, randomised 1055 patients to an EP combination (3-weekly epirubicin 90 mg/m² and paclitaxel 175 mg/m²) or to FEC (5-fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m²).[28]

Because of missing data the paclitaxel study could not be included in our pooled analysis (Figure 2.2). Disease-free survival of the pooled analysis including all seven docetaxel substitution studies showed a hazard ratio of 0.85 (95% CI 0.78 to 0.93), but there was a trend to heterogeneity (P=0.11). Heterogeneity disappeared when studies were analysed separately in four categories: TC versus AC (hazard ratio 0.67; 95% CI 0.49 to 0.92), TAC versus FAC (hazard ratio 0.70; 95% CI 0.59 to 0.84), AT versus AC (hazard ratio 0.96; 95% CI 0.84 to 1.09) and AT versus FEC (hazard ratio 0.89; 95% CI 0.76 to 1.05). We conclude that substitution by docetaxel is superior to conventionally dosed anthracycline-regimens if used in combination with cyclophosphamide, but not if used as anthracycline-taxane doublet by omitting cyclophosphamide.

Taxanes in sequence, partly substituting AC or FEC

Eight studies assessed in nine comparisons the impact of taxanes in sequence, partly substituting an anthracycline-combination regimen (Table 2.2)[9,30-36]

Two studies compared eight cycles of chemotherapy consisting of sequential docetaxel (100 mg/m^2 every three weeks) following or preceding an epirubicin/cyclophosphamide combination (EC→T or T→EC) with six cycles of FEC triplet.[30,31] Both studies demonstrated an improvement in disease-free survival in favour of the taxane arm. Nitz et al, also showed a significant improvement in overall survival for the taxane arm.[31]

Four studies assessed the efficacy of two to four cycles of taxane in sequence with three to four cycles of 5-fluorouracil, anthracycline and cyclophosphamide (FAC), as compared to six or eight cycles FAC, that is, the UK-TACT, PACS 01, GEICAM 9906 and an MDACC trial (Table 2.2).[9,32-34] The pooled hazard ratio of disease-free survival of these four studies is 0.85 (95% CI 0.75 to 0.96) in favour of taxanes (Figure 2.3).

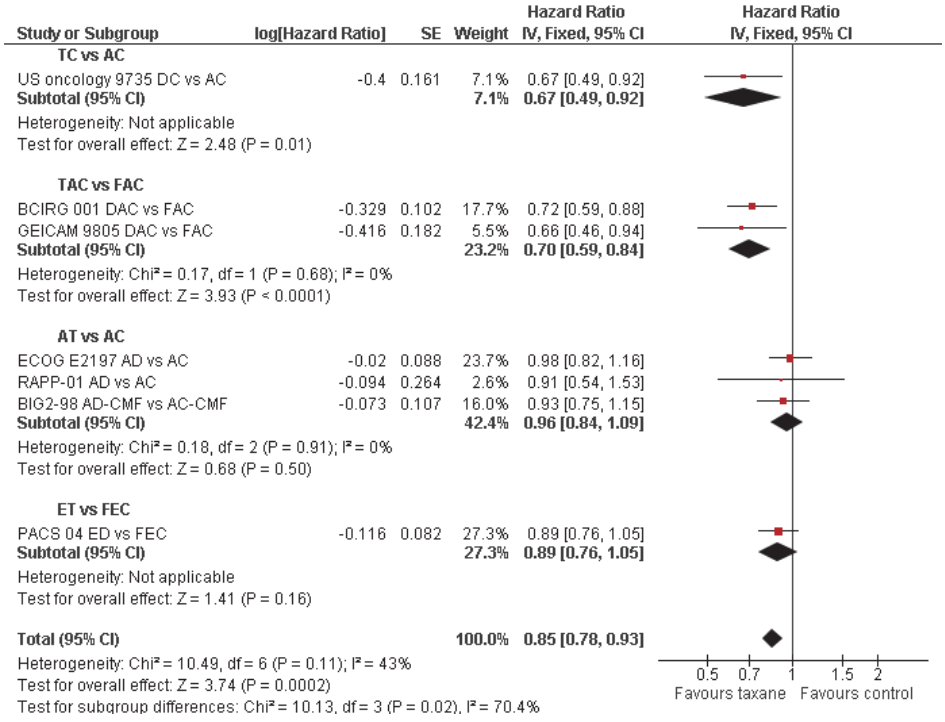

Figure 2.2 Taxane substitution in early breast cancer, hazard ratio of disease-free survival. Subgroups are based by the way the taxanes are used.

A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, M=methotrexate, P=paclitaxel, T=taxane.

Two studies compared a sequential anthracycline-taxane regimen with a dose-intensive anthracycline-containing poly-chemotherapy Canadian CEF (5-fluorouracil 500 mg/m^2 d1 + d8, epirubicin 60 mg/m^2 d1 + d8 and oral cyclophosphamide 75 mg/m^2 d1 - 14, 4-weekly).[35,36] In the German ADEBAR study, a sequential epirubicin/cyclophosphamide (90/600 mg/m^2)-docetaxel (100 mg/m^2) chemotherapy regimen was compared to Canadian CEF, with no significant difference in survival between the two treatment arms.[35] In the MA21 study, two or three-weekly anthracyclines in combination with cyclophosphamide (AC) and followed by 3 cycles of paclitaxel was compared with Canadian CEF.[36] The AC (60/600 mg/m^2)–P (175 mg/m^2) arm with 3-weekly paclitaxel was significantly inferior to Canadian CEF in terms of disease-free survival. The dose-dense epirubicin arm, with 3-weekly paclitaxel (EC120/830 mg/m^2 q2w–P 175 mg/m^2) was not significantly different from Canadian CEF. In our pooled analysis (Figure 2.3), including the studies on taxane in sequence, partly substituting conventionally dosed anthracycline-based therapy, we observed a trend for improved disease-free survival for taxanes (hazard ratio of 0.89 (95% CI 0.77 to 1.03; heterogeneity p=0.001). When excluding the trials using

Canadian CEF from the pooled analysis, because of the statistical heterogeneity, taxanes significantly improved disease-free survival, with a hazard ratio of 0.82 (95% CI 0.73 to 0.91; heterogeneity p=0.16), and for overall survival, with a hazard ratio of 0.77 (95% CI 0.66 to 0.90; data not further shown). We conclude that use of taxanes in sequence, partly substituting conventionally dosed chemotherapy, results in a superior outcome. However, results also show that by increasing anthracycline dose in the comparator regimen, the difference in outcome disappears.

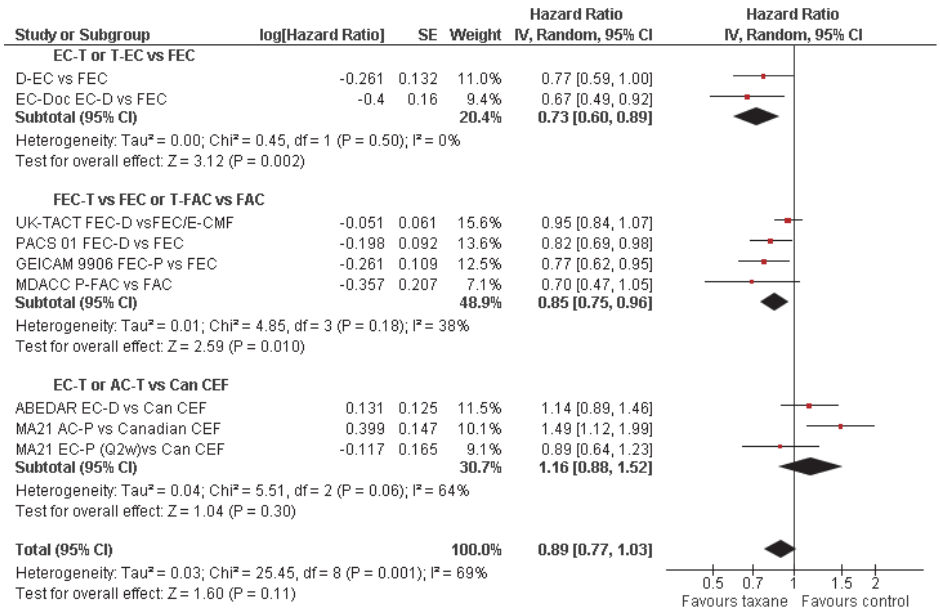

Figure 2.3 Taxanes in sequence, partly substituting AC or FEC in early breast cancer, hazard ratio of disease-free survival. Subgroups are based by the way the taxanes are used.
A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, M=methotrexate, P=paclitaxel, T=taxane.

Addition of taxanes: concurrently or sequentially to anthracyclines

In the ECTO trial a taxane was added concurrently to an anthracycline, followed by CMF (Table 2.2).[37] In total, 904 patients either received four cycles of adriamycin 75 mg/m^2 followed by four cycles of classical CMF or four cycles of adriamycin (60 mg/m^2) in combination with paclitaxel (200 mg/m^2), followed by four cycles of CMF. Patients treated with taxane combination chemotherapy had an improved 5-year disease-free survival, and a trend towards improved overall survival.

In five studies a taxane was given in sequence: either following an anthracycline (A→T vs A) or following a combination of an anthracycline and cyclophosphamide (AC→T vs AC), and next followed by CMF in three studies (table 2.2).[26,38-41] In the BIG 2-98 trial,

three cycles of docetaxel (100 mg/m^2) in sequence to three cycles of adriamycin (75 mg/m^2), and followed by three cycles of CMF, resulted in an improved 5-year disease-free survival when compared with the non-taxane control group four cycles of adriamycin (75 mg/m^2) followed by 3 cycles of CMF.[26] The TAXit-216 study with a more or less similar design, epirubicin (120 mg/m^2) followed by docetaxel (100 mg/m^2) followed by CMF, showed that with four additional docetaxel cycles, the 5-year disease-free survival showed an improvement of borderline significance.[38] In both the NSABP-B28 and the CALGB 9344 trial patients were treated with four cycles of AC or four cycles of AC followed by four cycles of 3-weekly paclitaxel at doses of 225 mg/m^2 and 175 mg/m^2, respectively (AC-P).[39,40] The dose of adriamycin was 60 mg/m^2 in the NSABP-B28 study, whereas different doses of adriamycin were used in the CALBG 9344 study, i.e. 60, 75 and 90 mg/m^2. Both trials showed a significant improvement in 5-year disease-free survival in favour of paclitaxel, but only in the CALBG 9344 study five-year overall survival was also significantly improved. The negative findings in the HeCOG study are based on a small number of events.[41]
The pooled analyses demonstrated that addition of taxanes, either concurrently or sequentially, improved disease-free survival and overall survival with a hazard ratio of 0.82 (95% CI 0.76 to 0.88) (Figure 2.4) and 0.85 (95% CI 0.76 to 0.94; data not further shown), respectively.

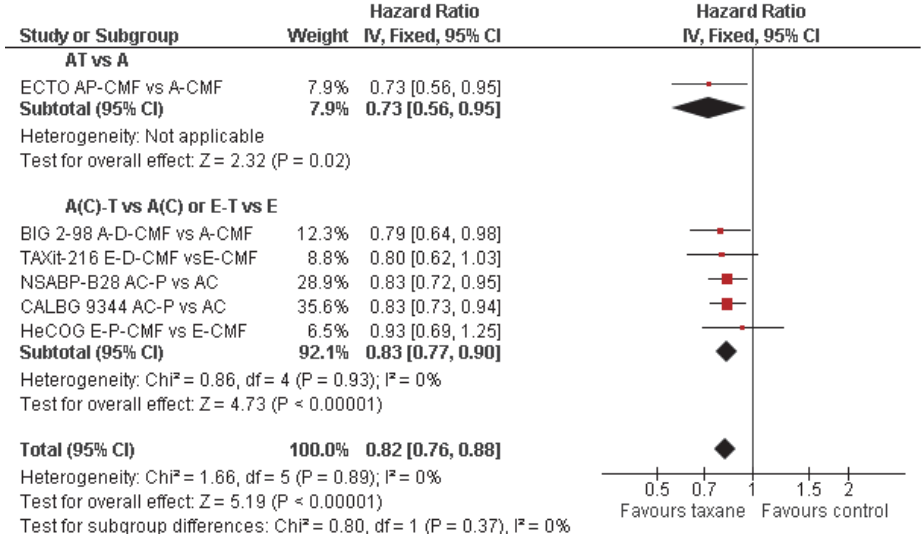

Figure 2.4 Taxane added concurrent or in sequence in early breast cancer, hazard ratio of disease-free survival. Subgroups are based by the way the taxanes are used.
A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, M=methotrexate, P=paclitaxel, T=taxane

Sequential versus concurrent use of anthracyclines and taxanes in metastatic and early breast cancer

Finally, we analysed the direct comparison of sequential versus concurrent use of taxanes (Tables 2.1 and 2.2).[20,26,42-44]

Conte *et al,* randomised patients to receive four cycles of epirubicin followed by four cycles of paclitaxel (E-P, 120-250 mg/m^2) or eight cycles of the combination (EP, 90/200 mg/m^2).[42] Alba *et al* randomised patients to receive three cycles of adriamycin followed by three cycles of docetaxel (A-T, 75-100 mg/m^2) or six cycles of the combination (AT, 50/75 mg/m^2).[20] In both trials of metastatic breast cancer no significant differences were found in response rate, time to progression or overall survival (Table 2.1). It is noted that these similar results were achieved with a lower cumulative dose in the sequential arms.

In two early breast cancer studies docetaxel was given in sequence to adriamycin (and CMF) or in sequence to a combination of adriamycin and cyclophosphamide and compared with adriamycin-docetaxel (Table 2.2).[26,43] In the pooled analysis of these two trials we found that disease-free survival was significantly improved, with a hazard ratio of 0.81 (95% CI 0.73 to 0.90; data not further shown) for the sequential arm. The higher efficacy of the sequential arm may be due to a higher number of chemotherapy cycles, use of full-dosed single agents per cycle (with comparable cumulative dose in the BIG 2-98 trial) and / or use of cyclophosphamide (in the NSABP-B30 trial).

In two early breast cancer studies docetaxel was either given in sequence following a combination of adriamycin and cyclophosphamide (AC-T; 60/600 – 100 mg/m^2) or concurrently (TAC; 75/50/500) (Table 2.2).[43,44] In the NSABP-B30, AC-T was compared with four cycles of TAC.[43] Patients treated with a sequential chemotherapy regimen showed a better 5-year disease-free survival and a trend toward better overall survival. The BCIRG 005 study used the same sequential regimen, but compared this with six instead of four cycles of TAC chemotherapy.[44] No difference in disease-free survival was seen. We conclude that sequential use of AC-T for a total of eight cycles is not superior to six cycles of TAC, but superior to four cycles of TAC. It should also be noted that in the BCIRG 005 trial the sequential use resulted in the same outcome as concurrent use of drugs, but with a lower cumulative dose of the chemotherapy.

Discussion

In our meta-analysis, we included 10 randomised trials that assessed the role of taxanes in the metastatic breast cancer setting, and 21 trials in the early breast cancer setting. We show that the use of taxanes did not improve overall survival in metastatic breast cancer trials (hazard ratio of 0.98; 95% CI 0.91-1.05), whereas it did so in early breast cancer (hazard ratio of 0.85; 95% CI 0.79 to 0.91). The primary goal of our review and meta-analysis was to determine whether the study design could account

for this differential outcome. For that purpose, we categorised the metastatic and early breast cancer trials by design. Such an approach has never been reported before. As a result, we noticed that in the majority of metastatic breast cancer studies taxanes were substituting other active cytotoxic drugs, mainly cyclophosphamide, whereas in early breast cancer studies taxanes were generally delivered in addition to a standard chemotherapy regimen. We conclude from our analyses that use of taxanes instead of other active drugs explains the lack of overall survival benefit in metastatic breast cancer trials. Further, our results suggest that cyclophosphamide is an important drug in the treatment of breast cancer, being as effective as taxanes.

In early breast cancer, many studies focused on the impact of taxanes when delivered at full dose in addition to anthracyclines, instead of substitution. Sequential use of taxanes after or before an anthracycline-containing regimen resulted in an improved disease-free survival at a hazard ratio of 0.82 (95% CI 0.76 to 0.88) and in an improved overall survival at a hazard ratio of 0.85 (95% CI 0.76 to 0.95), which is also in line with the recently updated overview of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG).[45] Apart from full-dose, the higher number of cycles could have contributed to the increased efficacy of sequential regimens. A drug that has shown comparable efficacy to a well-known effective drug in the metastatic breast cancer setting may thus still be worthwhile to test as an additional treatment in the early breast cancer setting. Moreover, with the availability of more drugs, and thus more lines of treatment in the metastatic setting, a general increment in overall survival can be expected for patients in daily practice. This emphasises the dilemma on how to interpret a so-called negative survival outcome of a randomised trial in the metastatic setting. In the end, not approving such a drug for introduction in daily practice may impede small steps of improvement over the years, both in metastatic and early breast cancer patients.

In metastatic breast cancer, the pooled hazard ratio for overall survival was 1.02 (95% CI 0.89 to 1.16) when substituting single-agent anthracyclines by single-agent taxanes. But, outcome in the individual studies was highly dependent of the anthracycline dose: 3-weekly (inferior) paclitaxel was less effective than conventionally-dosed adriamycin, and comparable effective as low-dose adriamycin. Anthracycline-taxane doublets were shown to have a similar effect as anthracycline-cyclophosphamide doublets with a hazard ratio for overall survival of 1.03 (95% CI 0.92 to 1.15), but were superior when compared to the triplet 5-fluorouracil, adriamycin, cyclophosphamide at a hazard ratio of 0.69 (95% CI 0.56 to 0.85). In the triplet, adriamycin dose was 50 mg/m^2, whereas in the doublet this was 60 mg/m^2. This and other studies indicate the importance of an adequate anthracycline dose.[45] Indeed, when looking at early breast cancer studies, a further improvement of the outcome for high-dose anthracyclines over taxanes was suggested, once the control arm FEC was delivered at a higher anthracycline dose. However, an intensified anthracycline regimen, like Canadian CEF,

is associated with increased risks for long term toxicities, like cardiotoxicity and leukaemia.[35,36] For that reason, most physicians prefer a taxane containing regimen over an intensified anthracycline regimen. We conclude that if anthracyclines are indicated, an adequate dose of at least 60 mg/m^2 adriamycin or 90 mg/m^2 epi-adriamycin should be used if applied in a combination regimen, which is also concluded in a recent review on anthracyclines.[46]

We also assessed whether the dose and choice of taxane, docetaxel or paclitaxel, could account for the differential outcome in metastatic versus early breast cancer setting. In two studies paclitaxel was used at a, not standard, high-dose of 220 mg/m^2 and 250 mg/m^2.[19,42] According to current knowledge such high doses will not have improved efficacy, whereas it probably has caused increased toxicity. Only one (adjuvant) paclitaxel study used weekly paclitaxel, whereas the others used 3-weekly paclitaxel. 3-weekly paclitaxel is highly unfortunate, because this is nowadays considered to be an inferior schedule for paclitaxel.[47-49] In contrast, all docetaxel studies were performed with 3-weekly docetaxel, which is the most optimal docetaxel schedule.[50-52] Indeed, when pooling the docetaxel trials in the metastatic breast cancer setting, overall survival showed a trend for improved overall survival for docetaxel regimens, at a hazard ratio of 0.88 (95% CI 0.76 to 1.01) which was not seen for the paclitaxel studies. However, the choice of taxane could not completely explain the differential impact of taxane results because in the adjuvant setting a survival benefit was seen with paclitaxel (hazard ratio 0.85 (95% CI 0.77 to 0.94) to a similar degree as with docetaxel.

The final question we addressed was the one regarding the most optimal use of taxanes: sequential or concurrent. In the few metastatic studies that are reported on this issue, survival was comparable for both strategies.[20,42] In early breast cancer sequential treatment seemed to be slightly superior in terms of disease-free survival in three out of four studies.[26,43] However, in one study use of cyclophosphamide in the experimental-arm may have been of relevance for outcome, and in two studies the higher number of cycles.[26,43] The BCIRG 005 study showed that sequential treatment has similar efficacy, but with a lower cumulative dose.[44] This suggests that sequential delivery of cytotoxic drugs at an optimal dose per cycle is relatively more effective. The lower cumulative dose might be particularly attractive for the anthracyclines with possible lower risk for long term toxicities.

In the studies on metastatic breast cancer included in our analyses, nearly all patients received first-line treatment, but some received second-line treatment. Mauri *et al.* have shown that most regimens in metastatic breast cancer have very similar efficacy profiles (less than 5% difference in hazard ratio) as first- and subsequent line therapies,[53] which supports our strategy to perform analyses irrespective of line of treatment. Interestingly, they have investigated the role of taxanes in metastatic

breast cancer from a different point of view than we did, and with a different methodology. They performed a multiple-treatments meta-analysis and calculated hazard ratios for each treatment category relative to monotherapy with old agents. They were able to show that newer regimens indeed achieved further benefits: for example for single-drug taxane a hazard ratio of 0.67 (95% CI 0.55-0.81) was found as compared to older drugs.

In our study, we showed that in a head-to-head comparison one drug may not be superior to another active drug with respect to efficacy endpoints. It is stressed that this may be the case in an unselected patient population, but not necessarily in a more molecular defined subpopulation. In the near future, we may be able to define more and more molecular subtypes. It is reasonable to believe that due to breast cancer heterogeneity drug-efficacy may vary per subtype and that conclusions of today may not hold true tomorrow. For instance, the hormone receptor positive luminal A breast cancer subtype responds less well to neoadjuvant chemotherapy than HER2-like and basal-like tumours, an observation we also see in our laboratory in models of breast cancer.[54] However, this implies that in trials with potential overrepresentation of luminal A tumours, patients drug-efficacy may be overall underestimated. Perhaps we must accept then the challenge to retest 'old' drugs for each and every subtype, therefore future clinical research should re-consider inclusion criteria and adapt to those based on disease biology rather than on anatomically defined risk factors. Similarly, the current observations in classical drugs may not simply be translated to biological drugs. In many instances, the biology of metastatic disease is different from early disease, which may explain why certain drugs (mainly the so called biological drugs) may have different efficacy in these two settings. Therefore, also the concept needs to be challenged that if a drug does not work in the metastatic setting it can immediately be inferred that it will also not work in the adjuvant setting (and vice-versa).

Our analysis is based on published data rather than on individual patient data, which may be considered a limitation of our analysis. For example, different definitions of progression-free and disease-free survival may hamper inter-trial comparisons. On the other hand, within each trial the same definition has been used for both treatment arms. Therefore, the risk of bias due to inter-trial differences in our pooled analysis is probably low.

The primary aim of our study was to explain the different outcome of taxanes in metastatic versus early breast cancer trials. We did not include analyses on toxicity or on costs. However, it is obvious that in the decision-making of reimbursement costs should be taken into account and for the individual patient benefits should always be weighed against side effects for every treatment that is considered to be an option. The most important side effects of taxanes are alopecia, febrile neutropenia,

neuropathy, allergic reactions and fluid retention, whereas for anthracyclines these are alopecia, febrile neutropenia and cardiotoxicity. All these side effects may have an impact on quality of life, also depending on the effect of treatment on tumour related complaints.

We conclude that taxanes are effective cytotoxic drugs in breast cancer treatment leading to an overall survival benefit in early breast cancer. If overall survival would have been the principal endpoint in metastatic breast cancer studies, the drug probably would not have been approved in current days. However, the negative results of taxanes in metastatic breast cancer studies seem to be caused by both the design of the randomized trial and the chosen comparator. For future drug approvals, we recommend to take these issues into account. Re-assessment of outcomes of studies of drugs both assessed in metastatic and early disease setting provides a new tool in a better understanding of this complex topic.

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Appendix 2

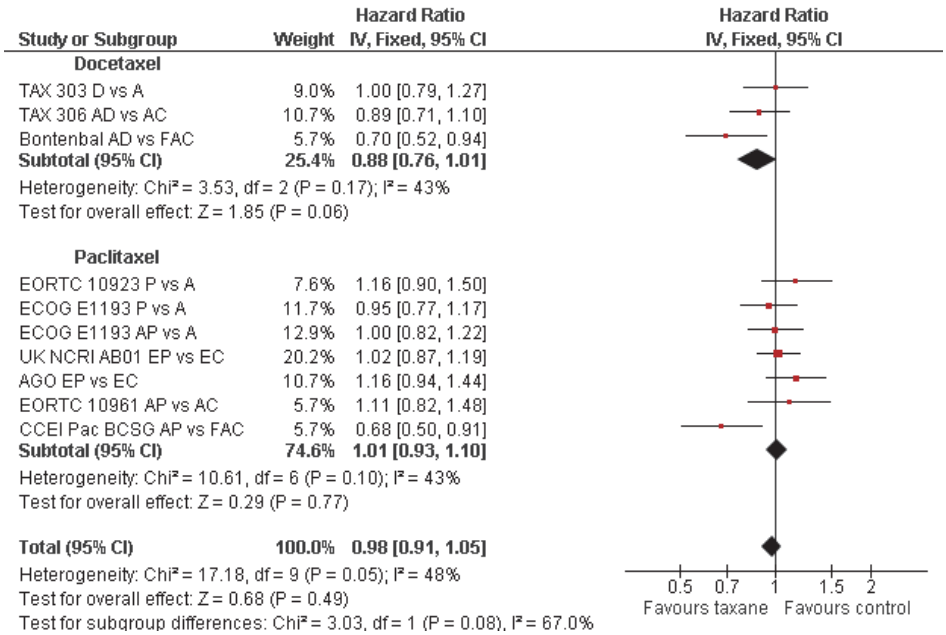

Figure S2.A Chemotherapy regimens with versus without taxane in metastatic breast cancer, hazard ratio of overall survival. Subgroups are based on the type of taxane used.
A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, M=methotrexate, P=paclitaxel

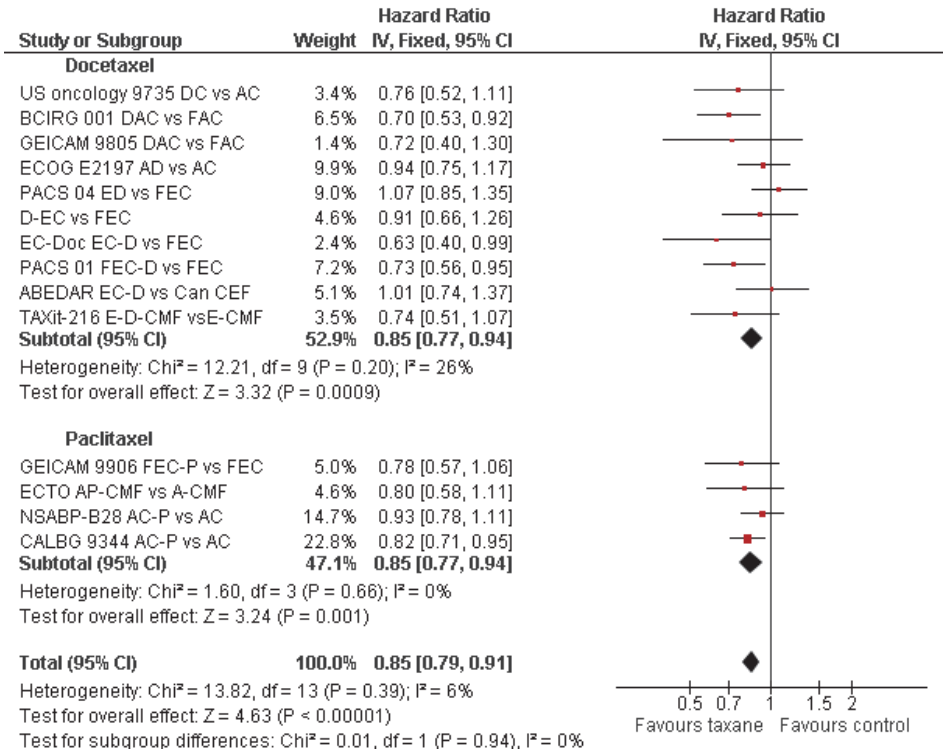

Figure S2.B Chemotherapy regimens with versus without taxane in early breast cancer, hazard ratio of overall survival. Subgroups are based on the type of taxane used.
A=adriamycin, C=cyclophosphamide, CI=confidential interval, D=docetaxel, E=epirubicin, F=5-fluorouracil, M=methotrexate, P=paclitaxel.

Chapter 3

Doxorubicin/cyclophosphamide with concurrent versus sequential docetaxel as neoadjuvant treatment in patients with breast cancer

Birgit EPJ Vriens, Maureen JB Aarts, Bart de Vries, Saskia M van Gastel, Jaap Wals, Tineke J Smilde, Laurence JC van Warmerdam, Maaike de Boer, Dick Johan van Spronsen, George F Borm, Vivianne CG Tjan-Heijnen

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Abstract

Background

This study was designed to determine whether delivering neo-adjuvant chemotherapy at a higher dose in a shorter period of time improves outcome of breast cancer patients.

Patients and methods

Women with newly diagnosed breast cancer were randomly assigned to neoadjuvant chemotherapy of four cycles of doxorubicin and cyclophosphamide followed by four cycles of docetaxel (AC 60/600 – T 100 mg/m^2) or six cycles of TAC (75/50/500 mg/m^2) every 3 weeks. The primary endpoint was the pathologic complete response (pCR) rate, defined as no invasive tumour present in the breast.

Results

In total, 201 patients were included. Baseline characteristics were well balanced. AC-T resulted in pCR in 21% and TAC in 16% of patients (odds ratio 1.44 (95% confidence interval (CI) 0.67-3.10). AC-T without primary granulocyte-colony stimulating factor (G-CSF) prophylaxis was associated with more febrile neutropenia compared to TAC with primary G-CSF prophylaxis (23% versus 9%), and with more grade 3/4 sensory neuropathy (5% versus 0%).

Conclusion

With a higher cumulative dose for the concurrent arm, no differences were observed between the two treatment arms with respect to pCR rate. The differential toxicity profile could partly be explained by different use of primary G-CSF prophylaxis.

Introduction

Neoadjuvant chemotherapy has become the standard of care in patients with locally advanced or borderline resectable breast cancer.[1] Interest has developed in extending this approach to patients with less advanced disease. Neoadjuvant chemotherapy allows observation of clinical response to systemic treatment, has the potential to down-stage the primary tumour which may facilitate breast conserving therapy, and bears the opportunity of down staging the axilla obviating the need of axillary treatment in some patients.[2] In a trial setting, a neoadjuvant approach is attractive as with far less patients a more rapid outcome is available in comparison to adjuvant trials.

Currently, anthracyclines, cyclophosphamide and taxanes are considered to represent the most potent drugs in breast cancer.[3,4] The NSABP-B28 and the CALGB-9344 trials were the first and largest studies to show a significant improvement in 5-year disease-free survival (72% versus 76% and 65% versus 70%, respectively), and the CALGB-9344 also in 5-year overall survival, for the addition of 3-weekly paclitaxel in sequence to four cycles of adjuvant doxorubicin and cyclophosphamide.[5,6] Subsequently, the upfront combination of docetaxel with doxorubicin and cyclophosphamide (TAC) was shown to outperform the combination of 5-fluorouracil, doxorubicin and cyclo-phosphamide (FAC) as adjuvant treatment in node-positive and node-negative breast cancer patients.[7–9]

In the neoadjuvant setting, the sequential administration of docetaxel after anthracycline-based therapy versus the anthracycline regimen alone was studied in two randomised studies.[10,11] In the Aberdeen study, patients received four cycles of anthracycline-based chemotherapy; responders were randomised to receive another four cycles of anthracycline-based chemotherapy or four cycles of docetaxel.[10] Switch to docetaxel showed a substantial improvement in response rate and an increased rate of breast conserving therapy. The NSABP-B27 trial also showed that addition of docetaxel after neoadjuvant anthracycline-based chemotherapy improved outcome with a significant increase in the pathological complete response (pCR) rate (14% versus 26%).[11,12] Furthermore, relapse-free survival was moderately improved in the neoadjuvant docetaxel-containing arm.

Hence, both the upfront combination of docetaxel with anthracyclines and cyclo-phosphamide and the sequential use of docetaxel have shown to improve breast cancer outcome. In this study, we hypothesised that the planned chemotherapy dose and dose-intensity may be a critical factor for predicting outcome. This is supported by the hypothesis that delivering chemotherapy within a shorter time frame prevents tumour outgrowth and development of resistance and should thus be more efficacious than sequential regimens in which the chemotherapy is given in a larger time frame.[13]

Patients and methods

Study design

This was a multicentre, open-label, phase III study in women with newly diagnosed breast cancer. Patients were randomly assigned to neoadjuvant sequential chemotherapy or combination chemotherapy consisting of doxorubicin, cyclophosphamide and docetaxel.
Patients provided written informed consent before enrolment. The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The study was approved by the Ethics Committee in agreement with the Dutch law code for medical research on humans.

Patient eligibility

Women eligible for the study were between 18 and 70 years with a Karnofsky performance score of at least 70%. Eligible patients had a primary tumour size of 3 cm or more and/or presence of positive regional lymph nodes. Patients were required to have optimal haematologic, renal and liver functions. No prior history of malignancy or anti-tumour therapy was allowed.

Treatment

Patients in the AC-T arm received four 3-weekly cycles of doxorubicin and cyclophosphamide at a dose of 60 and 600 mg/m^2, respectively, followed by four 3-weekly cycles of docetaxel (100 mg/m^2). Patients who were assigned to TAC chemotherapy received six cycles of doxorubicin, cyclophosphamide and docetaxel at doses of 75, 500 and 50 mg/m^2, respectively, every 3 weeks. All drugs were administered intravenously. During TAC chemotherapy G-CSF (6 mg pegfilgrastim) was recommended as primary prophylaxis. Chemotherapy dose was modified for haematologic and non-haematologic grade III/IV toxicities.
Next, patients underwent breast surgery and if indicated radiotherapy, endocrine therapy and/or trastuzumab.

Assessments

Mammography, ultrasound of breast and axillary lymph nodes and magnetic resonance imaging (MRI) breast had to be carried out before start of chemotherapy.
Biopsies of the primary tumour were taken for histological analysis, including oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 receptor (HER2) status. In case of clinically suspected axillary lymph nodes, fine needle aspiration was recommended. In case of a clinically negative axilla, a sentinel node procedure was recommended. All patients underwent radiologic evaluations to exclude distant metastases.

The clinical tumour response was rated using the response evaluation criteria in solid tumours (RECIST 1.0).[14] Adverse events were evaluated according to Common Toxicity Criteria version 3.0 (National Cancer Institute).
The pathologic response was locally assessed and in addition centrally reviewed by a breast-dedicated pathologist (BdV) using the Miller and Payne grading system.[15]

Statistics

The primary outcome measure was the pCR rate to neoadjuvant chemotherapy, which was defined as no invasive tumour cells present in the breast.
We hypothesised that the TAC regimen, with a higher total dose delivered in an overall shorter time period (that is a higher dose-intensity for the entire schedule), might be more effective than AC followed by T. To achieve 80% power at a 5% level of significance for the detection of a difference in proportion of pCR of 16% in AC-T arm versus 34% in the TAC arm a total of 180 eligible patients were required. Taking a 10% ineligibility rate into account, it was decided to enrol a total of 200 patients.
Secondary endpoints included the pCR rate of the axillary nodes, clinical response rates, disease-free and overall survival, the delivered chemotherapy dose and dose-intensity and the tolerability (grade 3/4 CTC toxicities) of both chemotherapy regimens. The pCR rate of the axillary nodes was assessed in patients who had clinically positive lymph nodes at start of chemotherapy (preferably with cytological proof), that were not removed or identified by the sentinel node procedure.
Randomisation procedure was done by inclusion in strata in a 1:1 ratio to a sequential or concomitant taxane regimen. Stratification was for cT-classification (T2 versus T3 + T4), cN-classification (cN0 versus cN+), hormone receptor status (positive versus negative) and HER2 status (positive versus negative).
All main analyses were done on the intent-to-treat-population. The primary parameter was analysed using the Cochran–Mantel–Haenszel test, whereas the logrank test was used to analyse disease free and overall survival. Other categorical parameters were analysed using the uncorrected chi-squared test. To investigate the influence of possible predictors (or risk factors) logistic regression was used.
All analyses were done using two-sided tests and level of significance 5%. The corresponding 95% confidence intervals (95% CI) were given whenever appropriate.

Results

Patient characteristics and treatment

From February 2006 through April 2009, a total of 202 women from 21 hospitals in the Netherlands were enrolled in this study. The majority of patients were included in the period from April 2008 through April 2009.

After randomisation, treatment data of one patient were not available. Therefore, this analysis is based on 201 patients, of whom 100 were allocated to the AC-T arm and 101 to the TAC arm (Figure 3.1)

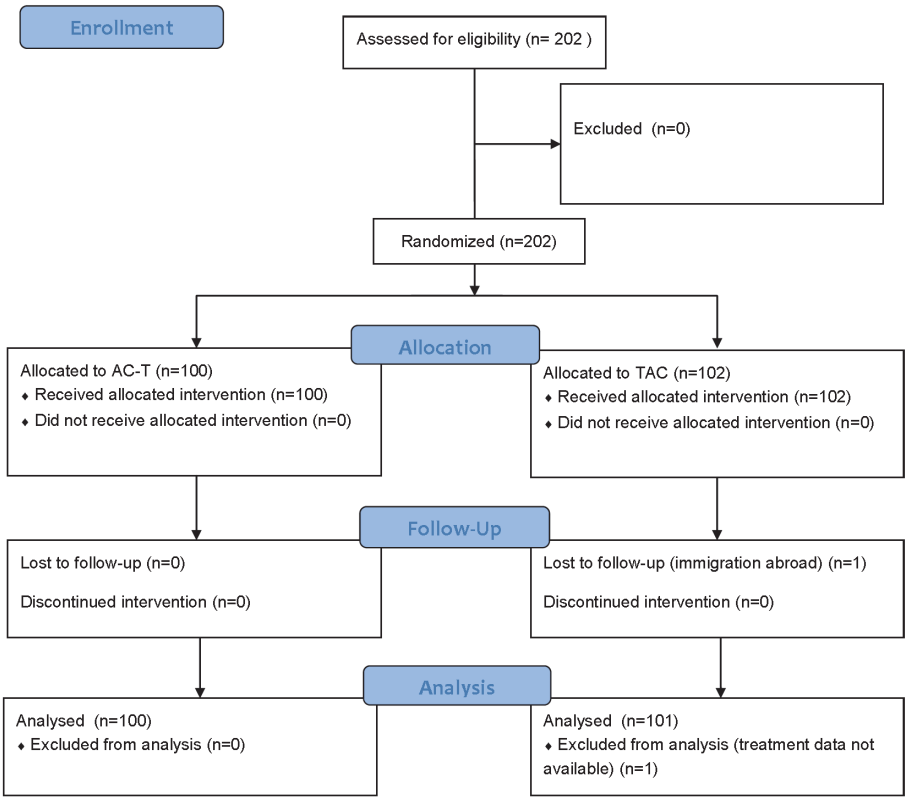

Figure 3.1 Consort diagram.

The two treatment arms were well balanced in terms of demographics and tumour characteristics (Table 3.1). Of 82 patients with a clinical negative axilla, 73% underwent a sentinel node procedure before start of neoadjuvant chemotherapy. Of 119 patients with a clinically suspected axilla, nodal metastases were confirmed in 79 patients by fine needle aspiration cytology.

Most patients received the planned number of chemotherapy cycles. Dose reductions and treatment delays were infrequent. In 99% of patients surgery was performed after completing the last chemotherapy and most patients received additional therapies after surgery (Table 3.2A).

Table 3.1 Baseline patient and tumour characteristics.

	AC-T (*n*=100) %	TAC (*n*=101) %
Age, years		
Median	49	49
Range	27-70	24-68
Initial tumour status		
cT1	2	5
cT2	49	45
cT3	34	30
cT4	15	20
Initial nodal status		
cN0	24	26
cN1	71	68
cN2	4	4
cN3	1	2
Receptor status		
ER and/or PR positive	66	65
HER2 positive	25	15
ER and/or PR and HER2 positive	14	8
ER/PR and HER2 negative	24	27

A, doxorubicin; C, cyclophosphamide; T, docetaxel; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Pathologic complete response rate

By central review, a pCR of the breast tumour was seen in 21% of 100 patients treated with AC-T and in 16% of 101 patients treated with TAC at an odds ratio of 1.44 (95% CI 0.67–3.10) (Figure 3.2A). Based on the local pathology reports, the pCR rates were 28% and 19%, respectively, with an odds ratio of 1.60 (95% CI 0.90–3.21).

In patients with a clinical positive axilla at start of chemotherapy, a pCR in axillary nodes was found in 19 of 60 patients (32%) treated with AC-T and in 12 of 59 patients (21%) treated with TAC with an odds ratio of 1.77 (95% CI 0.74–4.25). In this group a pCR in both breast and axilla was seen in 15% and 11%, respectively with an odds ratio of 1.53 (95% CI 0.48–4.86) (Table 3.2B).

Irrespective of treatment arm, patients with a triple negative tumour had the highest pCR rate (38%) (Figure 3.2B).

Table 3.2A pTN classification and interventions after neoadjuvant chemotherapy.

	AC-T (*n*=99) %	TAC (*n*=98) %
Tumour status		
ypT0	21	16
ypT1/T2	75	71
ypT3/4	4	12
Nodal status		
ypN0	49	42
ypN1	31	35
ypN2	14	16
ypN3	5	7
ypT0N0	17	10
Intervention after chemotherapy		
Surgical intervention		
No surgical intervention	1	3
Breast conservation	34	21
Mastectomy	65	77
Radiotherapy	85	80
First endocrine therapy		
Tamoxifen	48	56
Aromatase inhibitor	28	26
Trastuzumab	28	18

A, doxorubicin; C, cyclophosphamide; T, docetaxel.

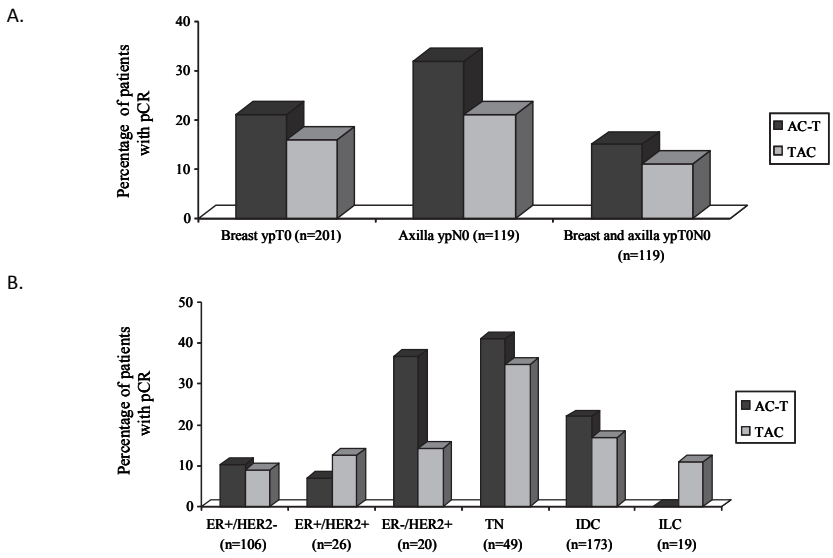

Figure 3.2 **A**. Percentage of patients with pCR in breast, axilla and combination in the two treatment arms AC-T and TAC. **B**. Percentage of patients with pCR in the breast in relation to the receptor status and morphology in the two treatment arms AC-T and TAC. pCR, pathologic complete response; A, doxorubicin; C, cyclophosphamide, T, docetaxel; ER, estrogen receptor; HER2+, human epidermal growth factor 2 receptor positive; TN, triple negative; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Table 3.2B ypTN classification patients with a clinical positive axilla at start of chemotherapy.

	AC-T (*n*=60) %	TAC (*n*=59) %
ypN0	32	21
ypT0N0	15	11

Clinical response rate

Most patients had a clinical response in the breast after treatment (Figure 3.3). Patients in the TAC arm with clinical stable disease halfway rarely had pCR after six cycles. Patients with a clinical complete response at the end of chemotherapy had a pCR in 58% and 41% of patients for AC-T and TAC, respectively. For patients with clinical partial response these rates were 19% and 15%, respectively, and for patients with clinical stable disease 0% in both arms.

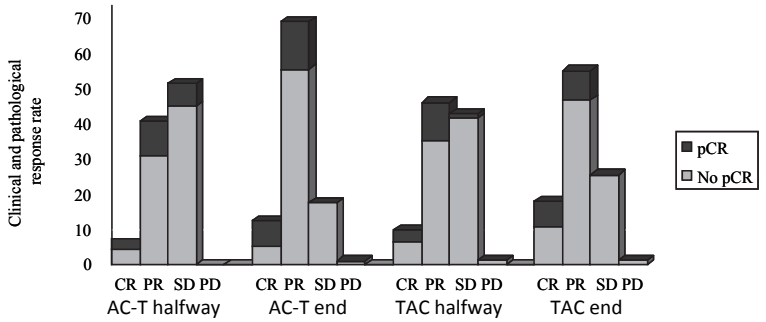

Figure 3.3 Clinical response rate halfway and at the end of chemotherapy per treatment regime, related to presence or absence of pCR in the breast at surgery. pCR, pathologic complete response; A, doxorubicin; C, cyclophosphamide, T, docetaxel; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Disease-free and overall survival

The 3-year disease-free survival was 88% for the AC-T arm and 75% for the TAC arm (*P*=0.21). The 3-year overall survival was 94% and 78%, respectively (*P*=0.11) (data not further shown).

Toxicity of neoadjuvant chemotherapy

Patients in the AC-T arm had more frequently grade 3/4 toxicities as compared to the TAC arm. This was mostly due to the higher incidence of neutropenic fever with AC-T, 23%, as compared to 9% with TAC (Table 3.3). Of note, most of these febrile events during AC-T occurred during docetaxel mono-chemotherapy (82%). The patients in the AC-T arm did not receive primary G-CSF prophylaxis. During treatment, G-CSF prophylaxis was started in 12% of patients treated with AC-T. In the TAC arm 99% of

the patients started with G-CSF prophylaxis during the first chemotherapy cycle. Of patients with febrile neutropenia while on TAC, 33% stopped the G-CSF prophylaxis previously.
The most common grade 3–4 non-haematologic toxicities were non-neutropenic fever (AC-T 5%; TAC 6%), sensory neuropathy (AC-T 5%; TAC 0%) and fatigue (AC-T 9%; TAC 4%) (Table 3.3).

Table 3.3 Incidence of CTCAE grade 3 and 4.

	AC-T (*n*=100)		TAC (*n*=101)
	AC (%)	T (%)	(%)
Hematologic toxicity (grade 3-4)			
Anaemia	1	0	1
Neutropenia	17	3	0
Trombocytopenia	2	3	0
Non-Hematologic toxicity (grade 3-4)			
Febrile neutropenia	4	19	9
Fever/infection without neutropenia	1	4	6
Nausea	3	0	0
Vomiting	1	0	0
Diarrhoea	1	3	0
Mucositis/Stomatitis	0	3	1
Sensory neuropathy	0	5	0
Allergic reaction	0	1	1
Fatigue	3	7	4
Muscle or bone pain	0	2	2
Pain (other than muscle/bone pain)	2	2	0
Thrombosis/embolism (grade 2-4)	0	1	2

A, doxorubicin; C, cyclophosphamide; T, docetaxel; CTCAE, Common Terminology Criteria Adverse Events.

Discussion

Both the sequential use of docetaxel after anthracyclines and cyclophosphamide (AC-T) and the upfront combination of docetaxel with anthracyclines and cyclophosphamide (TAC) have shown to improve early breast cancer outcome. This present randomised study of the Dutch Breast Cancer Trialists' Group (BOOG) was designed to determine whether higher dosed neoadjuvant chemotherapy, delivered in a shorter time frame, would provide the best outcome. In our study no significant efficacy difference was found between the sequential and upfront combination regimens, with a pCR of the breast tumour in 21% of patients treated with AC-T and in 16% of patients treated with TAC chemotherapy. Patients with ER positive tumours had the lowest pCR rate, whereas patients with triple negative tumours had the highest pCR rate. Clinical response rates showed to be related to pathologic response rates.

Other neoadjuvant trials studying taxane-containing chemotherapy show comparable pCR rates (Table 3.4). Of note, baseline patient and tumour characteristics and definition of pCR have a large impact on reported pCR rates. Studying other neoadjuvant breast cancer studies with pCR as primary endpoint, it becomes clear that there is no consensus on the definition of pCR. Absence of residual invasive disease after neoadjuvant chemotherapy in the breast, lymph nodes or both, including or excluding the *in situ* component is used (Table 3.4).[27] In the GeparQuinto trial pCR rates vary from 30% to 49% depending on definition of pCR used (Table 3.4).[26]

Table 3.4 Different used definitions of pCR in other trials.

Study	N	cTN-status	Scheme	pCR definition	% pCR (ypTN)
GeparQuinto[16]	1948	$cT_{1-4}N_{0/+}$	EC-D+Bev	ypT_0N_0	18.4
			EC-D		14.9
GeparQuinto[16]	1948	$cT_{1-4}N_{0/+}$	EC-D+Bev	$ypT_0N_{0/+}$	20.5
			EC-D		16.5
GeparQuinto[16]	1948	$cT_{1-4}N_{0/+}$	EC-D+Bev	$ypT_{0/is}N_0$	21.7
			EC-D		18.3
GeparQuinto[16]	1948	$cT_{1-4}N_{0/+}$	EC-D+Bev	$ypT_{0/is}N_{0/+}$	24.6
			EC-D		20.6
Prepare[17]	733	$cT_{2-4}N_{0/+}$	E(dd)-P(dd)-CMF	$ypT_0N_{0/+}$	18.7
			EC-P		13.2
GeparQuattro[18]	1421	$cT_{1-4}N_{0/+}$	EC-D	$ypT_0N_{0/+}$	22.3
			EC-DX		19.5
			EC-D-X		22.3
Gepartrio[19]	1390	$cT_{1-4}N_{0/+}$	6 cycles DAC	ypT_0N_0	21
			8 cycles DAC		23.5
Gepartrio[19]	1390	$cT_{1-4}N_{0/+}$	6 cycles DAC	$ypT_0N_{0/+}$	23.7
			8 cycles DAC		25.4
ACCOGS[20]	342	$cT_{\geq 3cm}N_{0/+}$	AC	$ypT_0N_{0/+}$	15
			AD		16
ACCOGS[20]	342	$cT_{\geq 3cm}N_{0/+}$	AC	$ypT_{0/is}N_{0/+}$	24
			AD		21
ACCOGS[20]	342	$cT_{\geq 3cm}N_{0/+}$	AC	$ypT_{0/is}N_0$	16
			AD		12
Aberdeen trial[10]	168	$cT_{\geq 3cm}N_{0/+}$	CVAP	$ypT_{0/is}N_{0/+}$	15.4
			CVAP-D		16.3
MDACC[21]	221	$cT_{1-3}N_{0/+}$	Pw-FEC100	$ypT_{0/is}N_0$	16.4
			D+Xd1-14-FEC100		19.8
Untch[22]	668	$cT_{1-4}N_{0/+}$	EP	$ypT_{0/is}N_{0/+}$	18
			EPdd		10
Untch[22]	668	$cT_{1-4}N_{0/+}$	EP	ypT_0N_0	12
			EPdd		6
$Geparduo_{23}$	913	$cT_{2-3}N_{0/+}$	AC-D	ypT_0N_0	14.3
			AD		7
NSABP-B27[11]	2411	$cT_{1c-3}N_{0/+}$	AC	$ypT_{0/is}N_{0/+}$	13.7
			AC-D		26.1

Table 3.4 (continued)

Study	N	cTN-status	Scheme	pCR definition	% pCR (ypTN)
HER2 targeted therapy					
HannaH study[24]	596	$cT_{1-4}N_{0/+}$	D-FEC+Trsc	$ypT_{0/is}N_{0/+}$	45.4
			D-FEC+Triv		40.7
HannaH study[24]	596	$cT_{1-4}N_{0/+}$	D-FEC+Trsc	$ypT_{0/is}N_0$	39.2
			D-FEC+Triv		34.2
NeoALTTO[25]	355	$cT_{2-4}N_{0/+}$	L-P	$ypT_{0/is}N_{0/+}$	24.7
			Tr-P		29.5
			L/Tr -P		51.3
NeoALTTO[25]	355	$cT_2N_{0/+}$	L-P	$ypT_{0/is}N_0$	20.0
			Tr-P		27.6
			L/Tr -P		46.8
GeparQuinto[26]	620	$cT_{1-4}N_{0/+}$	ECTr-DTr	ypT_0N_0	30.3
			ECL-DL		22.7
GeparQuinto[26]	620	$cT_{1-4}N_{0/+}$	ECTr-DTr	$ypT_0N_{0/+}$	34.2
			ECL-DL		26
GeparQuinto[26]	620	$cT_{1-4}N_{0/+}$	ECTr-DTr	$ypT_{0/is}N_0$	44.6
			ECL-DL		30.2
GeparQuinto[26]	620	$cT_{1-4}N_{0/+}$	ECTr-DTr	$ypT_{0/is}N_{0/+}$	49.8
			ECL-DL		35.7

A, doxorubicin; Bev, bevacizumab; C, cyclophosphamide; D, docetaxel; dd, dose dense; E, epirubicin; F, 5-fluorouracil; L, lapatinib; M, methotrexaat; P, paclitaxel; pCR, pathologic Complete Response; P, prednisolon; Tr, trastuzumab; Triv, trastuzumab intravenous; Trsc, trastuzumab subcutaneous; V, vincristine; w, weekly; X, capecitabine.

For calculating pCR rates of axillary nodes it seems not correct to include patients with clinically negative nodes or patients in whom positive nodes have been removed by a sentinel node procedure. Therefore, for pCR of the lymph nodes we included only patients with clinical positive lymph nodes (preferably with cytological proof) at start of chemotherapy that were not removed by the sentinel node procedure. In most trials, all patients are included in the denominator, hence, leading to (incorrect) low axillary pCR rates.

So far, only three early breast cancer trials compared sequential versus concurrent use of taxanes, all in the adjuvant setting.[28–30] In the BIG 2-98 trial, sequential A-T for a total of six cycles after CMF was compared with concurrent doxorubicin-docetaxel (AT) for four cycles, resulting in a hazard ratio of 0.83 (95% CI 0.69–1.00) for 5-year disease-free survival in favour of the sequential arm.[28] In the NSABP-B30 trial, AC-T for eight cycles compared to AT for four cycles, showed an improved 5-year disease-free survival for the AC-T arm, with a hazard ratio of 0.80 (95% CI 0.70–0.91).[29] In this trial, AC-T for eight cycles was also compared to 4 TAC, again showing an improved 5-year disease-free survival for the sequential arm with a hazard ratio of 0.83 (95% CI 0.73-0.95).[29] In contrast, in the BCIRG-005 trial, eight cycles of AC-T did not improve 5-year disease-free survival when compared to six cycles of TAC (hazard ratio of 1.00; 95% CI 0.86–1.16).[30] In our neo-adjuvant trial, we also compared eight cycles of AC-T with six cycles of TAC. Similar to our observation, others also have shown that sequential treatment at a lower dose per cycle in a modestly higher number of cycles

compared to concurrent chemotherapy (6 versus 4, and 8 versus 6) seems equally effective, which from a cost-effective viewpoint may be attractive.

The toxicity of both regimes was manageable. In our study febrile neutropenia was the most frequent side effect. It was seen in 23% of patients treated with AC-T and in 9% treated with TAC, whereas in the BCIRG 005 study these figures were 8% and 18%, respectively.[30] Different use of primary G-CSF prophylaxis probably explains the observed difference. In our study, 99% of TAC patients started with G-CSF prophylaxis during the first chemotherapy cycle, compared to only 17% of TAC patients in the BCIRG 005 study. In both trials none of the patients in the AC-T arm received primary G-CSF prophylaxis. We noticed that 83% of febrile neutropenic events in the AC-T arm occurred during docetaxel monotherapy, i.e. in 19% of patients on docetaxel. Interestingly, others reported, that this high rate was not observed when docetaxel was administered upfront before AC chemotherapy.[31] The mechanism remains unclear. To prevent this side effect, one might consider reversing the sequential treatment order, to reduce the docetaxel dose or to offer primary G-CSF prophylaxis during docetaxel monotherapy. The international guidelines do recommend primary G-CSF prophylaxis during chemotherapy if the risk of febrile neutropenia is more than 20%.[32] We observed no toxic deaths. In our study no left ventricular ejection fraction (LVEF) was done routinely, in contrast to the BCIRG 001 study (TAC versus FAC).[33] Annually in years 5–10 after treatment the LVEF was measured to explore long term cardiac effects. They conclude that a substantial percentage (16%) of patients had a decrease in LVEF, probably caused by anthracycline therapy. This may be an advantage of sequential therapy, because of a lower cumulative dose of anthracyclines.

Nowadays, most would prefer to give trastuzumab sequentially after anthracyclines and concurrently with taxanes, also in the neo-adjuvant setting.[34,35] At that time, guidelines recommended to administer trastuzumab after surgery. Moreover, our trial design prevented imbalance between treatment arms as safety data on concurrent trastuzumab-TAC were lacking. This might be considered a limitation of our study. We tested in an unplanned analysis, whether exclusion of the HER2 positive group would impact the study outcome. This was not the case (pCR 22% for AC-T and 16% for TAC).

In conclusion, it becomes evident that both AC-T and TAC are potent regimens currently available as (neo)adjuvant chemotherapy of breast cancer. Both treatment schedules have manageable toxicities. From our study results we argue there are some advantages for using AC-T over TAC, because of the lower cumulative dose per agent which makes it the most cost-effective approach, and also because sequential treatment results in a quick recognition of the *in vivo* response per drug, offering a rapid switch to another drug in case of lack of response.

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Chapter 4

Improved survival for sequentially as opposed to concurrently delivered neoadjuvant chemotherapy in non-metastatic breast cancer

Birgit EPJ Vriens, Ingeborg JH Vriens, Maureen JB Aarts, Saskia M van Gastel, Franchette WPJ van den Berkmortel, Tineke J Smilde, Laurence JC van Warmerdam, Dick Johan van Spronsen, Petronella GM Peer, Maaike de Boer, Vivianne CG Tjan-Heijnen on behalf of the Breast Cancer Trialists' Group of the Netherlands (BOOG)

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Abstract

Purpose

The INTENS study was designed to determine whether delivering neoadjuvant chemotherapy at a higher dose in a shorter period of time improves outcome of breast cancer patients.

Methods

Women with newly diagnosed breast cancer were randomly assigned to neoadjuvant chemotherapy consisting of four cycles of doxorubicin and cyclophosphamide followed by four cycles of docetaxel (AC 60/600 - T 100 mg/m^2) or six cycles of TAC as triplet chemotherapy (75/50/500 mg/m^2) every 3 weeks. The primary outcome was the pathologic complete response (pCR), with disease free and overall survival as secondary endpoints.

Results

In total, 201 patients were included. The pCR rates were 28% for patients treated with AC-T and 19% for patients treated with TAC, with an odds ratio of 1·60 (95%CI 0·90-3·21). With a median follow-up of 6 years (range 0.04-8.41 years) the five-year disease-free survival was 81% for patients treated with sequentially AC-T and 71% for patients treated with concurrent triplet TAC chemotherapy with a stratified hazard ratio (HR) of 0.50 (95% CI 0.29-0.86). Five-year overall survival was 84% versus 76%, respectively with a stratified HR of 0.55 (95% CI 0.29-1.03).

Conclusions

No differences were observed between the two treatment arms with respect to pCR rate, but the sequentially delivered chemotherapy outperformed the triplet combination chemotherapy in terms of survival, despite a lower cumulative dose per agent.

Introduction

It is accepted worldwide that taxanes should somehow be incorporated in the (neo)adjuvant treatment of breast cancer patients at increased risk of relapse. The most optimal strategy is however, still under investigation. Previously, we reported the breast pathological complete response (pCR) results from our Dutch phase III breast cancer study (the INTENS trial) comparing sequential versus concurrent use of taxanes in addition to doxorubicin and cyclophosphamide.[1]

To support accelerated approval, pCR in breast cancer is formally approved by the Food and Drug Administration as a surrogate endpoint of neoadjuvant chemotherapy trials for efficacy and to prognosticate long-term outcomes, especially in high risk patients (triple negative, HER2 (human epidermal growth factor receptor) positive).[2] Apparently, there is an interplay between treatment efficacy and tumour biology resulting in differential outcome patterns, as was shown in more recent neoadjuvant trials.[3-5] The prognostic value of pCR and long term outcome seemed to be strongest in patients with more aggressive subtypes.[3-5]

In the present analysis of the INTENS trial, we report the disease-free and overall survival rates by treatment arm, presence or absence of pCR and the effect of treatment per breast cancer subtype.

Patients and methods

The study design, patient characteristics and pCR results have been reported before.[1]

Study design

In short, the INTENS trial is a Dutch phase III study in which patients were randomly allocated to neoadjuvant chemotherapy in a sequential schedule consisting of four cycles of doxorubicin and cyclophosphamide followed by four 3-weekly cycles of docetaxel (AC-T; 60, 600 and 100 mg/m^2, respectively) or six 3-weekly cycles of a concurrent schedule consisting of the same drugs at a different dose per cycle (TAC; 75, 50 and 500 mg/m^2, respectively) and different cumulative dose and dose-intensity per drug for the entire schedule. At the time, HER2-targeted therapy was used as adjuvant treatment, also in patients with HER2-positive disease who were treated with neoadjuvant chemotherapy. The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

Patients

Patients with non-metastatic breast cancer and a Karnofsky Performance Score of at least 70 with a clinical tumour size of at least 3 cm and/or positive regional lymph

nodes were eligible. A total of 201 assessable patients were included from February 2006 through April 2009 from 21 hospitals in the Netherlands.

Study endpoints and statistical analyses

The primary outcome measure of the Dutch INTENS study was the pCR rate after neoadjuvant chemotherapy, defined as postoperative absence of invasive tumour in the breast. To achieve 80% power at a 5% level of significance for the detection of a difference in proportion of pCR of 16% versus 34% a total of 180 eligible patients were required. These percentages were based on results from the Aberdeen trial.[6] Taking a 10% drop out into account, it was decided to enrol a total of 200 patients. The results on the primary endpoint have been reported before.[1]

The primary objective of the current study was to determine the outcome in terms of disease free survival and overall survival according to treatment arm. Disease-free survival was defined as time from date of randomization until the date of occurrence of local or regional recurrence, contralateral or second primary ipsilateral breast cancers (including DCIS), or death of any cause. Overall survival was defined as time from the date of randomization until date of death of any cause. All patients still alive were censored at the date of last follow-up of each individual patient.

All analyses were done on the intent-to-treat population. Survival curves were obtained using the Kaplan-Meier method and tested for differences between two groups with the log-rank test.

The impact of treatment was expressed in a hazard rate ratio obtained in a Cox-model stratified for clinical tumour stage cT1-2 and cT3-4, clinically nodal status cN negative (cN0) and cN positive (cN+), receptor status (ER, estrogen; PR, progesterone) and HER2-status. The impact of treatment was also assessed in specific patients groups (age ≤50 years and age >50 years).

All reported P-values are two-sided and P-value <0.05 was considered statistically significant. The 95% confidence intervals (95% CI) were given whenever appropriate.

Results

Patient characteristics and treatment

Characteristics in terms of demographics and tumour were well-balanced across the groups (Appendix Table S4.1). At enrolment, median age was 49 years (range, 24-70 years). Many patients had rather extensive locoregional disease, with nearly 50% of them having cT3-4 tumours and 75% having clinical involvement of axillary lymph nodes, 66% of patients had ER and/or PR positive disease, 20% HER2-positive disease and 25% triple negative disease.[1]

Pathologic complete response rate

Based on the local pathology reports, the pCR rates were 28% and 19%, respectively, with an odds ratio of 1·60 (95%CI 0.90–3.21).[1]

Disease-free and overall survival per treatment arm and disease free survival per stratum

After a median follow-up of 6 years (range 0.04-8.41 years), 5-year disease-free survival was 81% for patients treated with sequential AC-T chemotherapy and 71% for patients treated with concurrent triplet TAC chemotherapy (log-rank *P*=0.015), resulting in a stratified HR of 0.50 (95% CI 0.29-0.86) in favour of the sequential treatment arm (Figure 4.1A). Five-year overall survival was 84% for the patients treated with AC-T chemotherapy versus 76% for those treated with TAC chemotherapy, resulting in a stratified HR of 0.55 (95% CI 0.29-1.03) (Figure 4.1B).
Sequential treatment provided the largest disease-free survival benefit in patients with cT1-2 tumours (HR 0.25; 95% CI 0.10-0.60) and hormone receptor positive / HER2 negative disease (HR 0.27; 95% CI 0.10-0.75) (Figure 4.2).

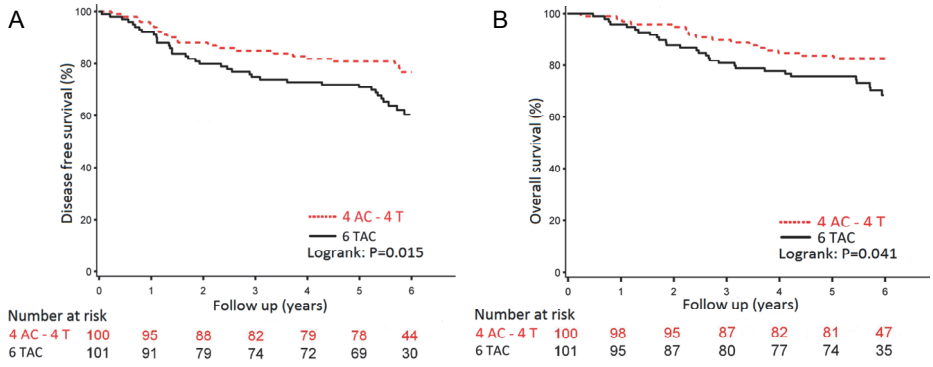

Figure 4.1 Disease-free (A) and Overall Survival (B) per neoadjuvant treatment arm: four cycles of doxorubicin and cyclophosphamide followed by four cycles of docetaxel (AC-T) or six cycles of concurrent triplet chemotherapy (TAC).

Outcome related to pCR in the breast

Five-year disease-free survival was 91% for those having a pCR in the breast and 71% for those without pCR (logrank *P* value 0.008) (Figure 4.3). In Figure 4.4, the results are shown by tumour subtype: hormone receptor positive / HER2 negative (Figure 4.4A: logrank *P* value=0.041), hormone receptor positive / HER2 positive (Figure 4.4B: logrank *P* value=0.212), hormone receptor negative / HER2 positive (Figure 4.4C:

logrank *P* value=0.055), hormone receptor negative / HER2 negative (Figure 4.4D: logrank *P* value=0.0046).
Comparable results were obtained for overall survival (not further shown).

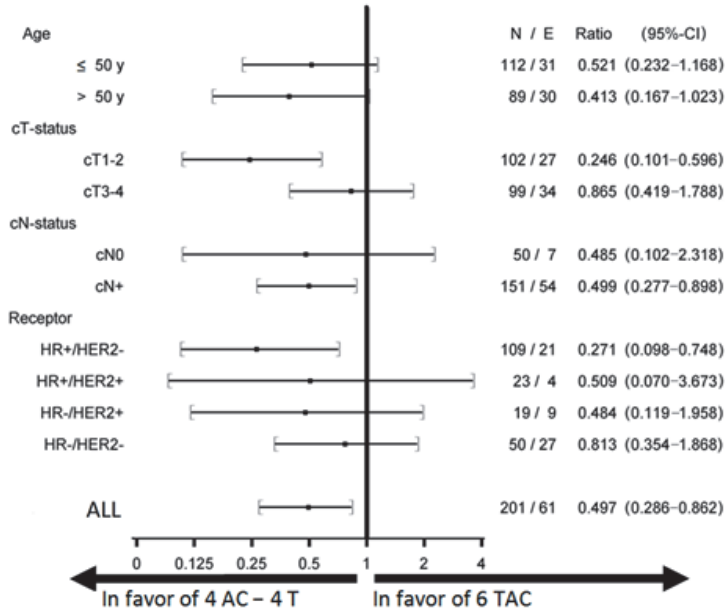

Figure 4.2 Forest plots comparing groups with AC –T and those treated with TAC neoadjuvant chemotherapy within various subsets for disease-free survival.
HR, hormone receptor; HER2, human epidermal growth factor receptor 2. The hazard ratios (HR) and their 95% CIs are obtained from the corresponding Cox proportional hazards models. HR<1 implies benefit from AC-T when compared to TAC.

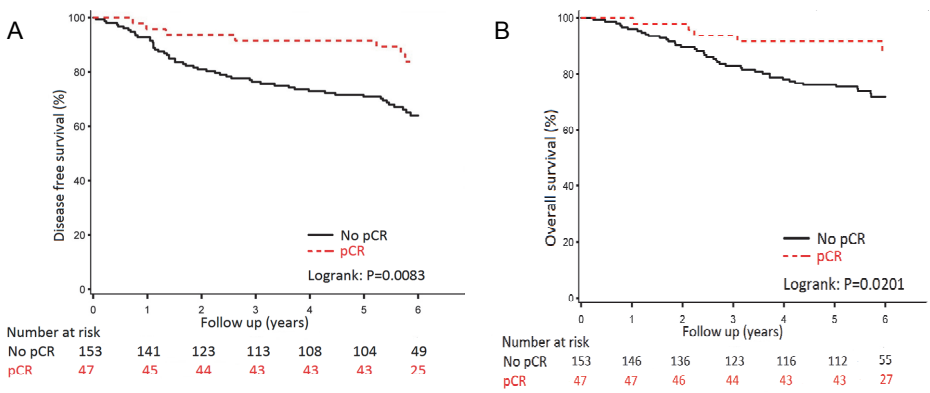

Figure 4.3 Disease-free Survival (A) and Overall survival (B) stratified by pCR for the overall population, irrespective of chemotherapy schedule.

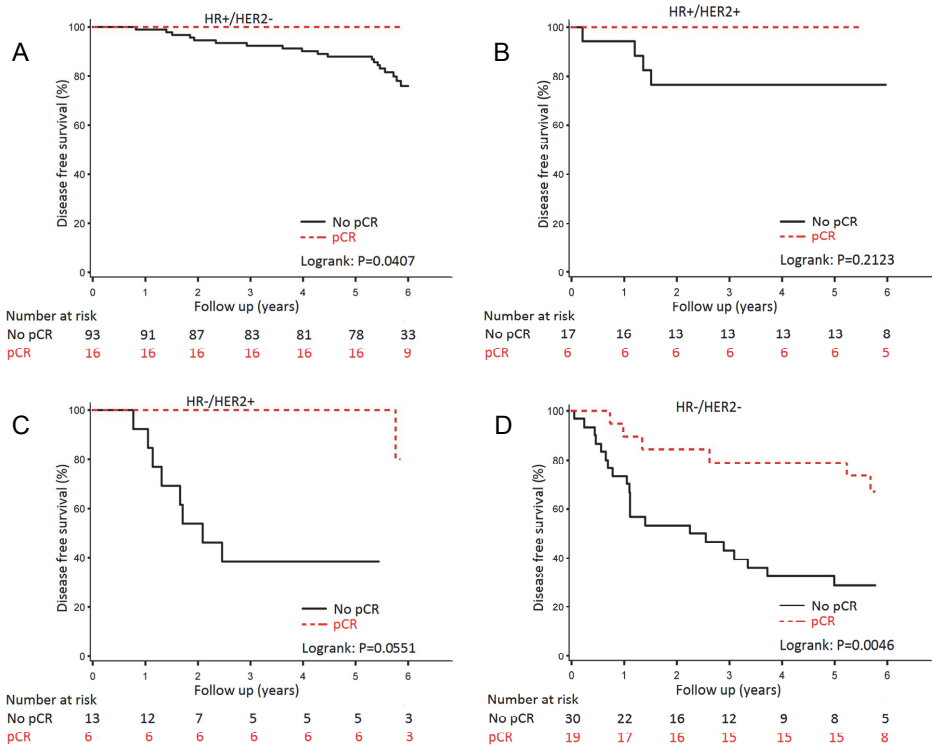

Figure 4.4 Disease-free survival stratified by pCR for the overall population per tumour subtype: hormone receptor (HR) / human epidermal growth factor receptor 2 (HER2), positive (+) and negative (-) subsets

Discussion

In this Dutch phase III neoadjuvant chemotherapy study, breast cancer patients were treated by either four cycles of doxorubicin and cyclophosphamide followed by four cycles of docetaxel (AC-T) or six cycles of concurrent triplet chemotherapy (TAC).[1] Previously, we reported the results on pCR based on the local pathology reports.[1] The pCR rates were 28% for patients treated with AC-T and 19% for patients treated with TAC, with an odds ratio of 1·60 (95%CI 0·90-3·21).

Now, we report the results after a median follow-up of six years, showing a superior disease-free and overall survival with sequentially delivered AC-T chemotherapy. Notably, all patient subgroups benefitted from the sequentially delivered eight cycles of treatment as compared to those treated with the triplet schedule, but the size of the benefit differed. Specifically patients with more favourable tumour characteristics benefitted the most, with a HR of 0.25-0.27 for disease-free survival. Moreover, we noticed that patients with a pCR and hormone receptor positive disease had an excellent 5-year disease-free survival.

Both the adjuvant breast cancer BIG 2-98 and the NSABP-B30 trials compared sequential versus concurrent use of taxanes resulting in a better disease-free survival for the sequential arm.[7,8] The BIG 2-98 trial compared amongst others six cycles of sequentially delivered A-T with four cycles of concurrently delivered AT chemotherapy, both after treatment with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) chemotherapy, resulting in a HR of 0.83 (95% CI 0.69-1.00) for disease-free survival in favour of the sequential treatment arm.[7] The NSABP-B30 trial compared AC-T for eight cycles to TAC for four cycles, again showing an improved disease-free survival for the sequential arm at a hazard ratio of 0.83 (95% CI 0.73-0.95).[8] In contrast, in the large adjuvant BCIRG-005 trial eight cycles of AC-T did not improve disease-free survival when compared to six cycles of TAC (HR 1.00; 95% CI 0.86-1.16).[9] In a recent meta-analysis, it was shown that patients with hormone receptor negative breast cancer may benefit from dose-dense chemotherapy, whereas those with hormone receptor positive breast cancer did not.[10] Apparently, number of chemotherapy cycles, dose per cycle and frequency of chemotherapy delivery all matter. In our study, each drug was given at a higher dose per cycle (dose-intensity), but at a lower cumulative dose at a 3-weekly interval. Taking all the results into account, we can at least conclude that sequentially delivered chemotherapy was always superior or comparable, but never inferior, to concurrently delivered triplet chemotherapy. Therefore, sequentially delivered chemotherapy may thus be the preferred treatment-strategy for non-metastatic breast cancer.

The patients in the NSABP-B18 study received a combination of doxorubicin and cyclophosphamide (AC) chemotherapy every 3 weeks.[11] The investigators of this trial were the first to show that patients with a pCR in the breast had an improved 5-year disease-free survival, suggesting its value as prognosticator.[11,12] Unexpectedly, an improvement in pCR rate by the addition of neoadjuvant docetaxel to AC chemotherapy did not result in a further improved overall survival in the NSABP-B27 trial.[13] In our study, addressing all biomarker subtypes, there is a non-significant pCR improvement for AC-T versus TAC. More than half of the patients in our study had ER positive and HER2 negative disease, this is the group excluded from the accelerated FDA approval. We conclude that in this population with the majority of patients with ER positive and HER2 negative disease a longer follow up period is necessarily to take conclusions about survival because pCR is a poor predictor of clinical benefit in this population and drug-efficacy may be overall underestimated. This is in line with other studies that despite the lower pCR rates in this population, patients with hormone receptor positive tumours nonetheless have a more favourable long-term prognosis.[5]
In our study patients with pCR in the breast had a significantly better survival than those without pCR (Figure 4.3). Although numbers per subgroup were too low to draw firm conclusions, it was noted that patients who did obtain a pCR in the breast with ER and/or HER2 positive disease had an excellent outcome (Figure 4.4). Of note, patients with HER2-positive disease (20% of all) received trastuzumab only as adjuvant

treatment, which may be considered a limitation of the study and may also explain the lack of significance between pCR and disease-free and overall survival for the HER2 positive subgroups. Patients with triple negative breast cancer and pCR in the breast had a significantly better outcome than those without pCR, 80% 5-year disease-free survival for patients who had a pCR versus 30% for those without pCR. These results are in line with the meta-analysis of Cortazar, although they reported a modest higher 5-year event-free survival of approximately 50% in patients with triple negative breast cancer with failure to achieve pCR.[5] The inferior outcome in our study may be explained by inclusion of more patients with aggressive characteristics as cT3-4 tumours and clinically node positive tumours.

The BIG 2-98 study showed that there is an association between presence of tumour infiltrating lymphocytes and prognosis.[14] Moreover, patients with HER2 positive disease with increased stromal lymphocytic infiltration had a larger benefit of anthracycline-based therapy compared to those receiving combination anthracycline-docetaxel therapy. These results suggest that specific chemotherapy schedules in specific breast tumours may trigger the immune system which contributes to treatment efficacy. Indeed, induced cancer cell death may increase the release of tumour-associated antigens with an increase in immune response, inducing tumour cell death.[15] Casares *et al.* observed this immunogenic effect of anthracycline-treated tumour cells in the absence of any adjuvant or co-stimulus.[16] In addition, one may hypothesize, that this immune effect may be more robust in the presence of tumour cells as is the case in the neoadjuvant setting, as opposed to the adjuvant setting. This might be an argument for choosing neoadjuvant chemotherapy, not using a too high dose of corticosteroids for at least the first doses of chemotherapy. More studies are needed to evaluate the interaction between immuno-surveillance and different types and timing of chemotherapy regimens.
Xing *et al.* suggested that dexamethasone could suppress immune response by enhancing programmed cell death protein 1 (PD-1).[17] PD-1 and programmed death ligand-1 (PD-L1) form the PD-1/PD-L1 complex, which plays a role in down regulating T-cell activity, which may result in faster tumour growth and poor prognosis in the clinical setting of anti-cancer therapy. Hence, it can be hypothesised that dexamethasone may have a negative influence on anti-cancer therapy efficacy through a negative impact on a tumour-related immune response. In our study different dosages of dexamethasone were used to prevent chemotherapy-related allergic reactions and other adverse effects in the two treatment arms. For docetaxel based treatment 8 mg of dexamethasone orally was given twice daily the day before, of and after docetaxel, whereas during AC treatment 8 mg of dexamethasone intravenously was given shortly before each cycle. Possibly the upfront use of high-dose corticosteroids during all cycles of concurrently versus in the last four cycles only of the sequentially treated arm might be a possible explanation for the difference in efficacy between the sequential versus concurrent use of taxanes.[1,9]

As we discussed earlier, the toxicity of both regimes was manageable.[1] The most important difference was the incidence of febrile neutropenia, which was reported in 23% of patients treated with the sequential regimen where primary prophylaxis with granulocyte colony-stimulating factor (G-CSF) was not part of the treatment protocol. This was sharply higher than the 9% rate of febrile neutropenia during TAC chemotherapy where primary G-CSF prophylaxis was mandatory. Most of these febrile events in the AC-T arm occurred during docetaxel mono-chemotherapy (82%). Other studies reported a risk of febrile neutropenia between 5-25%.[18,19] The European Organization for Research and Treatment of Cancer guidelines considered AC-T chemotherapy to have a high risk of febrile neutropenia (>20%).[20] For this reason, we would now routinely recommend the use of G-CSF prophylaxis for AC-T chemotherapy during the four cycles of docetaxel chemotherapy.[20] Hence, during AC-T chemotherapy G-CSF prophylaxis is only required in four cycles instead of six during TAC chemotherapy, which can be considered an advantage for sequentially delivered chemotherapy. More importantly, with AC-T a lower cumulative anthracycline dose can be delivered, which is very attractive as a possible detoriation of cardiac performance has already been reported in patients who received more than approximately 250-300 mg/m^2 of anthracycline.[21,22] Limiting G-CSF prophylaxis for four cycles during the docetaxel monotherapy saves costs without an increase of febrile neutropenia, together with the lower cumulative dose per agent compared to TAC chemotherapy makes the AC-T chemotherapy the most cost-effectiveness approach in times of rapidly rising healthcare costs. Finally, with AC-T chemotherapy a cold cap to prevent hair loss may be used. The Dutch Scalp Cooling Registry reported that of scalp-cooled patients; 63% of patients treated with AC-T chemotherapy did not wear a head cover during their last chemotherapy session in contrast to 8% of patients treated with TAC chemotherapy.[23]

To conclude, we showed that sequential AC-T neoadjuvant chemotherapy outperformed concurrent TAC chemotherapy in non-metastatic breast cancer patients, given at a lower cumulative dose.

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Appendix 4

Table S4.1 Baseline patient and tumour characteristics.

	AC-T (*N*=100) %	TAC (*N*=101) %
Age, years		
Median	49	49
Range	27-70	24-68
Initial tumour status		
cT1-cT2	51	50
cT3-4	49	50
Initial nodal status		
cN0	24	26
cN+	76	74
Receptor status		
HR+/HER2-	51	58
HR+/HER2+	15	8
HR-/HER2+	11	8
HR-/HER2-	23	27

A, doxorubicin; C, cyclophosphamide; T, docetaxel; HR, hormone receptor; HER2, human epidermal growth factor receptor 2

Chapter 5

Neutrophil recovery in breast cancer patients receiving docetaxel-containing chemotherapy with and without granulocyte colony-stimulating factor prophylaxis

Maureen J Aarts, Birgit E Vriens, Maaike de Boer, Frank P Peters, Caroline M Mandigers, M Wouter Dercksen, Jacqueline M Stouthard, J Tol, Laurence J van Warmerdam, Agnes J van de Wouw, Esther M Jacobs, Carin CD van der Rijt, Tineke J Smilde, Annette W van der Velden, Nelly Peer, Vivianne C Tjan-Heijnen

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Abstract

Objective

Primary outcome of the current study is, whether there is a protective effect of prior chemotherapy or of prior granulocyte colony-stimulating factor (G-CSF) on the next cycle blood cell counts.

Methods

Hematologic toxicity was evaluated, based on a randomised phase III study in breast cancer patients (n=167) with >20% risk of febrile neutropenia. The primary endpoint was the nadir blood cell counts for patients treated with G-CSF given during all six chemotherapy cycles or limited to the first two chemotherapy cycles only

Results

For the present analyses, 47 patients were eligible. In the G-CSF 1-6 arm, the median white blood cell count (WBC) and absolute neutrophil count (ANC) nadir slowly decreased from $10.8x10^9$/l in cycle 1 to $7.5x10^9$/l in cycle 6 and from $7.1x10^9$/l to $5.5x10^9$/l, respectively. The median WBC nadir in the G-CSF 1-2 arm decreased from $1.2x10^9$/l in cycle 3 to $0.9x10^9$/l in cycle 6 and the ANC nadir showed a grade 4 neutropenia of $0.1x10^9$/l in cycle 3-6. All patients had ANC recovery to normal levels (≥ $1.5x10^9$/l) without delay at day 1 of next cycle.

Conclusion

We conclude that there is no protective effect of prior G-CSF or prior chemotherapy use on nadir blood counts in subsequent cycles.

Introduction

An anthracycline-taxane chemotherapy regime is effective in breast cancer, but it is also very myelotoxic with a substantial risk of febrile neutropenia (FN).[1,2] The FN incidence of myelosuppressive chemotherapy regimes without primary G-CSF prophylaxis is reported to be highest in the first (two) chemotherapy cycles, with declining incidence thereafter.[1-4]
One might hypothesize that chemotherapy-induced myelosuppression leads to intrinsic hematopoietic growth factor production and as such protects against FN during later chemotherapy cycles. However, so far no relevant articles regarding this subject have been published.
At the same time, for patients who are treated with primary G-CSF prophylaxis (i.e. from the first cycle onwards) one may conclude that continuation of prophylaxis during later chemotherapy cycles may be less effective because of the lower baseline risk of FN in later chemotherapy cycles.[1,2] We performed, therefore, a phase III study in which breast cancer patients treated with myelosuppressive chemotherapy were randomised to G-CSF prophylaxis during all six chemotherapy cycles or to G-CSF prophylaxis limited to the first two chemotherapy cycles only.[5] In contrast to what we had expected, G-CSF use limited to the first two chemotherapy cycles was not effective, as in total 36% of patients developed FN compared to 10% in the control arm. Interestingly, we observed that the highest FN incidence in the experimental arm occurred in the third chemotherapy cycle (24% of patients), which was the first cycle without G-CSF prophylaxis. Remarkably, in the fourth to sixth chemotherapy cycle, the incidence of FN again declined to an average of 5%. This might possibly have been the result of secondary antibiotic prophylaxis (7% of patients), secondary G-CSF prophylaxis (17% of patients) or chemotherapy dose-modifications (8% of patients).

However, this observation may also be explained by a protective effect of prior chemotherapy or of prior G-CSF on the blood cell counts of the fourth to sixth chemotherapy cycle. If indeed this is the case, blood cell counts would gradually increase during subsequent chemotherapy cycles. We therefore studied nadir blood cell counts over the subsequent chemotherapy cycles in patients treated in the above mentioned phase III clinical trial. To prevent confounding, we excluded from our present analysis patients who developed FN event, received secondary G-CSF prophylaxis or chemotherapy dose reductions during later cycles.

Methods

Clinical phase III study

Full methods and results of the phase III study have been published before.[5,6] Patients were randomised to G-CSF prophylaxis during all six chemotherapy cycles or to G-CSF

prophylaxis limited to the first two chemotherapy cycles only. For the phase III study, breast cancer patients were eligible if treated with 3-weekly chemotherapy and being at risk of FN according to international guidelines. For example, TAC (docetaxel, adriamycin, cyclophosphamide) or AT (adriamycin, docetaxel) are chemotherapy regimes with more than 20% risk of FN; TC (docetaxel, cyclophosphamide) or docetaxel monotherapy have a 10-20% risk of FN and in the presence of one or more patient risk factors, risk of FN increases to more than 20%. Patients were required to have an absolute neutrophil count (ANC) >1.5 x 10^9/l, adequate renal and hepatic function, no signs of an active uncontrolled infection or other serious co-morbidity before enrolment.

Study design

We included the data from patients treated according to the aforementioned phase III study, and evaluated the hematologic nadir toxicity for all included patients per treatment arm.

Subsequently, we selected a subgroup of patients, in whom nadir blood cell counts throughout subsequent chemotherapy cycles were affected by a constant chemotherapy and G-CSF use. Hence, we excluded patients who developed FN, had chemotherapy dose-reductions or chemotherapy delays (more than 3 days from scheduled treatment) during treatment or who did not use G-CSF prophylaxis according to plan (either prematurely ceased G-CSF prophylaxis despite randomization to G-CSF prophylaxis during all six chemotherapy cycles, 'G-CSF 1-6 arm', or who had secondary G-CSF prophylaxis during cycles 3-6 in the 'G-CSF 1-2 arm' because of FN or other reason). In case of missing values in cycles 4-6, patients were considered not-assessable and therefore excluded.

Blood samples were taken on day 1 and nadir blood counts on day 10-14 of each cycle to assess myelotoxicity. In case of FN, daily complete blood cell counts were to be performed until recovery of ANC ≥ 0.5 x 10^9/l.

Study endpoints

The hematologic toxicity per cycle per treatment arm was evaluated, which was a pre-specified secondary endpoint of the randomised clinical phase III trial.[5] We analysed nadir white blood cell (WBC), neutrophil and platelet counts in patients treated with primary G-CSF prophylaxis during all chemotherapy cycles and of patients treated with G-CSF prophylaxis during cycles 1 and 2 only.

For the current study, the primary endpoint was the nadir blood cell counts in cycles 1-6 for the selected, eligible patients who received G-CSF prophylaxis in cycles 1-6 and the nadir blood cell counts in cycles 3-6 for the selected, eligible patients who per protocol did not receive G-CSF prophylaxis in cycles 3-6 (only G-CSF in cycles 1-2).

All analyses are descriptive in nature.

Results

Patients

In the clinical phase III trial, in total 167 patients were included. For the present research question, 32 patients in the G-CSF 1-6 arm were eligible and 15 patients in G-CSF 1-2 arm. We excluded patients because of FN (8 and 30 patients, respectively), because of chemotherapy delay or dose reductions (11 and 7, respectively) and because of G-CSF protocol violations (2 and 17, respectively). Other patients were excluded due to missing blood nadir values in one or more cycles. (Figure 5.1) Baseline characteristics are shown in Table 5.1.

Table 5.1 Baseline characteristics

	G-CSF given during cycles 1-6 in all patients in the Dutch two-to-six study n=84		G-CSF given during cycles 1-6 in patients selected for the present sub-study n=32		G-CSF given during cycles 1-2 in all patients in the Dutch two-to-six study N=83		G-CSF given during cycles 1-2 in patients selected for the present sub-study n=15	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Patient characteristics								
Median age, (range) years	50 (26-69)		56 (32-73)		50 (31-70)		58 (39-76)	
≤65 years	79	94	30	94	78	94	13	87
ECOG PS 0-1	81	96	32	100	81	98	15	100
(Neo-)adjuvant treatment setting	84	100	32	100	80	96	14	93
Primary antibiotics prophylaxis	34	40	17	53	36	43	5	33
Baseline blood counts, median (range)								
White blood cell count (x10^9/l)	7.7 (3.4-21.1)		7.0 (4-19.6)		7.6 (4.3-18.0)		6.8 (4.3-11.7)	
Absolute neutrophil count (x10^9/l)	4.8 (2.0-64.3)		4.4 (2.1-17.8)		4.5 (2.1-16.2)		3.9 (2.6-8.4)	
Platelet count (x10^9/l)	296 (129-541)		297 (143-541)		289 (160-544)		262 (160-544)	
Chemotherapy								
TAC	81	96	32	100	77	93	14	93
FEC-D	3	4	0	0	3	4	0	0
DOC	0	0	0	0	2	2	1	7
TC	0	0	0	0	1	1	0	0

G-CSF, granulocyte colony stimulating factor; PS, performance. Score; TAC, docetaxel, doxorubicin, cyclophosphamide; FEC-D, 5-Fluorouracil, epirubicin, cyclophosphamide-docetaxel; DOC, docetaxel: TC, docetaxel, cyclophosphamide

Nadir blood cell counts

In Table 5.2, it is shown that in the selected, eligible patients treated in the G-CSF 1-6 arm (n=32), median nadir WBC decreased from 10.8x10^9/l in cycle 1 to 7.5x10^9/l in cycle 6 and ANC from 7.1x10^9/l to 5.5x10^9/l. Similarly, nadir blood cell counts decreased during treatment in the G-CSF 1-2 arm when primary G-CSF prophylaxis was discontinued conform study protocol in cycle three and further: nadir WBC dropped from 1.2x10^9/l to 0.9x10^9/l and nadir ANC showed a persistent grade 4 neutropenia of 0.1x10^9/l in cycle 3-6. (Table 5.3).

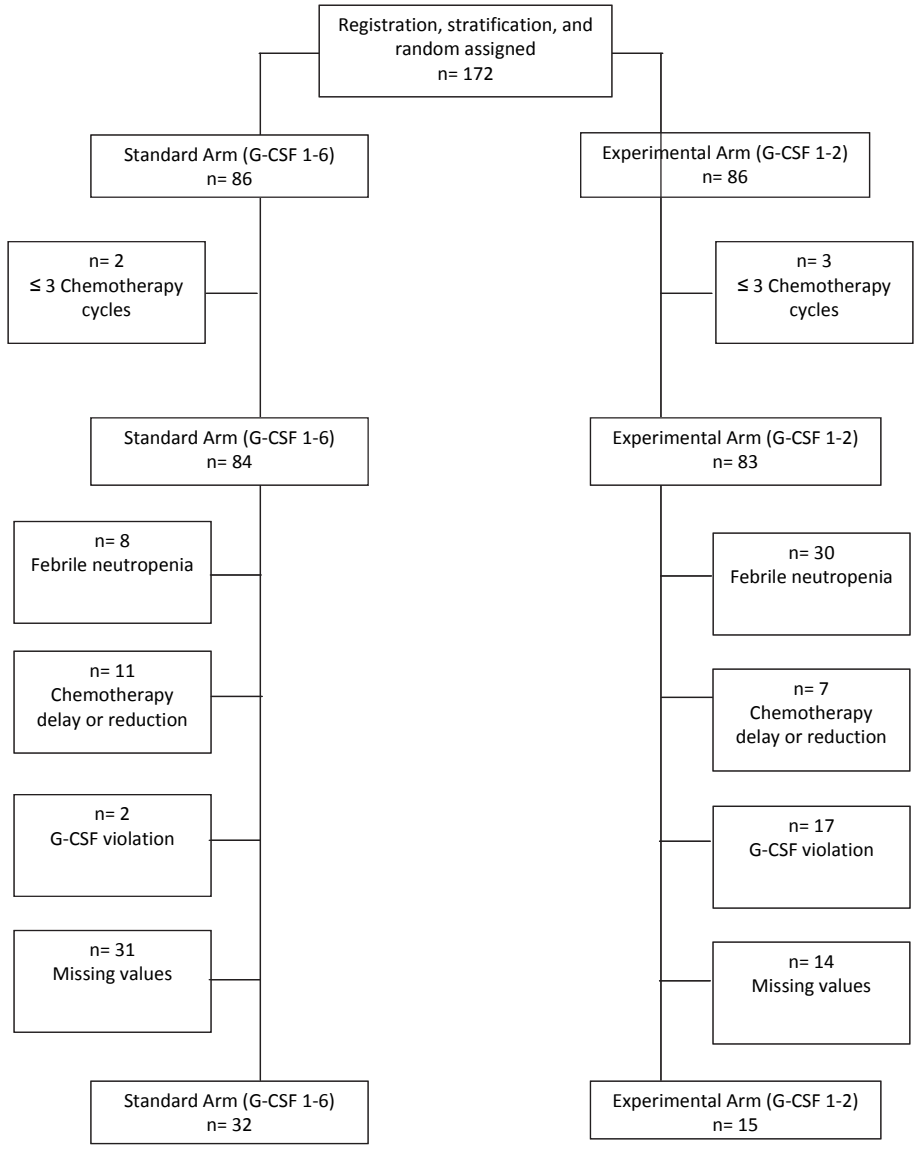

Figure 5.1 Consort flow diagram

Of note, when considering the total patient population irrespective of changes in chemotherapy or G-CSF administration, nadir blood cell counts in the G-CSF 1-6 arm gradually *decreased* during treatment, whereas in the G-CSF 1-2 arm nadir blood cell counts gradually *increased* from cycle 3 to 6: from $1.2x10^9$/l to $1.8x10^9$/l for WBC and from $0.1x10^9$/l to $0.5x10^9$/l for ANC (Tables 5.2 and 5.3).

Nadir platelet counts remained stable over the consecutive chemotherapy cycles (data not shown).

Table 5.2 Hematologic Toxicity G-CSF 1-6 arm all patients and subgroup*: Nadir values

	G-CSF given during cycles 1-6 in all patients in the Dutch two-to-six study N=84		G-CSF given during cycles 1-6 in patients selected for the present sub-study N=32	
	Median	IQR	Median	IQR
White blood cell count**				
Cycle 1	10.3	6.2-13.6	10.8	5.5-15.2
Cycle 2	10.1	8.1-12.3	10.1	7.9-15.4
Cycle 3	9.1	7.2-11.8	10.1	7.4-15.4
Cycle 4	9.1	7.4-11.8	9.4	5.1-12.0
Cycle 5	8.9	6.4-10.7	8.9	5.4-11.8
Cycle 6	7.8	5.3-9.3	7.5	3.4-9.5
Neutrophil count**				
Cycle 1	7.1	3.9-9.5	7.1	3.9-10.9
Cycle 2	6.7	5.3-8.6	7.5	5.7-11.0
Cycle 3	6.6	4.8-8.8	7.4	4.8-12.3
Cycle 4	6.6	5.2-8.4	7.0	3.4-9.6
Cycle 5	6.5	3.8-8.0	6.7	3.5-8.8
Cycle 6	5.3	3.3-6.8	5.5	2.5-7.4

* Subgroup; patients without FN, chemotherapy dose reductions or delay, G-CSF prophylaxis 'violation' or missing values cycle 3-6. **x10^9/l. G-CSF, granulocyte colony stimulating factor; IQR, interquartile range

Table 5.3 Hematologic Toxicity G-CSF 1-2 arm all patients and subgroup*: Nadir values

	G-CSF given during cycles 1-2 in all patients in the Dutch two-to-six study N=83		G-CSF given during cycles 1-2 in patients selected for the present sub-study N=15	
	Median	IQR	Median	IQR
White blood cell count**				
Cycle 3	1.2	0.7-1.8	1.2	0.8-1.4
Cycle 4	1.5	0.9-5.1	1.0	0.8-1.5
Cycle 5	1.4	1.0-6.3	1.2	0.8-1.4
Cycle 6	1.8	0.9-5.9	0.9	0.7-1.8
Neutrophil count**				
Cycle 3	0.1	0-0.5	0.1	0-0.2
Cycle 4	0.2	0.1-3.6	0.1	0-0.1
Cycle 5	0.2	0.1-4.3	0.1	0.03-0.1
Cycle 6	0.5	0.1-4.0	0.1	0.05-0.5

* Subgroup; patients without FN, chemotherapy dose reductions or delay, G-CSF prophylaxis 'violation' or missing values cycle 3-6; **x10^9/l. G-CSF, granulocyte colony stimulating factor; IQR, interquartile range

Day 1 blood cell counts

In patients in G-CSF 1-6 arm who used G-CSF prophylaxis during all chemotherapy cycles, the day-1 ANC values remained stable throughout cycles 3-6. The median day-1 ANC values were on average 5.8x10^9/l. In contrast, in the G-CSF 1-2 arm without

secondary G-CSF prophylaxis in cycles 3-6 as defined in the study protocol the median day-1 ANC slowly declined from cycles 3 to 6. (data not shown)

Discussion

FN is a serious side effect of cancer treatment.[3,4] For patients at risk (>20%) of FN, primary G-CSF prophylaxis is recommended as it reduces the risk of chemotherapy-induced FN.[7]
Previously, the risk of FN was presumed to be highest during the first chemotherapy cycles, but we showed that discontinuation of G-CSF prophylaxis after the first two chemotherapy cycles resulted in a rebound high FN incidence in the next chemotherapy cycles.[5] Apparently, a prior chemotherapy cycle in combination with G-CSF prophylaxis seemed not to be myeloprotective for a later chemotherapy cycle. In order to find support for this latter assumption, we looked more in detail to the hematologic toxicity in patients in whom in-patient comparisons were possible e.g. patients without chemotherapy dose modifications or changes in G-CSF prophylaxis during their treatment and without FN event. In our explorative analyses, we show that the median nadir WBC and ANC slowly decreased while continuing on G-CSF prophylaxis, and that the median ANC nadir remained steadily low in cycles 3-6 in those who did not receive G-CSF prophylaxis during these cycles. Hence, we conclude that prior G-CSF prophylaxis with pegfilgrastim during 3-weekly chemotherapy and prior chemotherapy has no myeloprotective effect on subsequent chemotherapy cycles.

Pegfilgrastim acts by self-regulated, neutrophil-mediated clearance.[8] In a dose-ranging pegfilgrastim study in breast cancer patients, it was demonstrated that the exposure to pegfilgrastim was lower in cycle 3 than in cycle 1, however the ANC nadir improved and the duration of neutropenia decreased in cycle 2 and the subsequent cycles, suggesting an expansion of neutrophil and neutrophil precursors mass in the later cycles that results in an increase in drug clearance.[9] This suggestion was supported by Invernizzi et al.,[10] who demonstrated not only an increase of ANC (evaluated at day 14 or later and immediately before next chemotherapy) but also an increase of immature myeloid cells in peripheral blood after the first chemotherapy cycle given with G-CSF support with a relatively constant level in the subsequent cycles. Without G-CSF support no increase of the neutrophil count immediately before chemotherapy occurs; the count was the lowest around two weeks after chemotherapy with return to baseline at day 1 of chemotherapy. Also, progenitor cells apoptosis was significantly higher in case no pegfilgrastim support was given compared with pegfilgrastim support.[10,11] So, increase of progenitor pool and decrease of apoptosis could be one factor explaining the differences in blood cell counts between both treatment arms. With this mechanism of action of G-CSF we would have expected an

increase of blood cell counts in the next chemotherapy cycle, however we observed a decrease.
The post-nadir ANC of ≥1.0x10^9/l is of relevance as it is seen as a surrogate marker threshold of pegfilgrastim clearance to subtherapeutic levels.[12,13] All our patients experienced an ANC recovery to normal levels (≥1.5x10^9/l) at day 1 of a new cycle while on G-CSF prophylaxis.

Previously, it was reported that the incidence of FN and of grade 4 neutropenia declines during later chemotherapy cycles, and that this cannot entirely be explained by use of secondary G-CSF prophylaxis or chemotherapy dose-reductions.[4,14] We also noticed a slight increase of nadir WBC and ANC during cycles 3-6 in the G-CSF 1-2 arm, but after exclusion of patients who re-initiated G-CSF prophylaxis (n=17) and of patients who had chemotherapy adjustments (n=7), we saw a persistent steady grade 4 neutropenia. Of note, we didn't exclude patients with primary antibiotics prophylaxis as its value in reducing grade 4 neutropenia is neglectable.

Factors that increase risk of neutropenia and FN are patient and treatment related. Patient risk factors are pretreatment, older age, lower weight, vascular comorbidity and baseline low WBC counts and higher bilirubin levels.[15-17] We couldn't identify any of these pre-treatment patient risk factors in our patient population. Of relevance, the occurrence of FN increases the risk for a new FN event, so we excluded the 38 patients that developed FN to exclude possible confounding. Several covariates in relation with interindividual variation in the time course of neutropenia have been investigated. The most relevant predictors for this interindividual variability were albumin, bilirubin, alkaline phosphatase, lactate dehydrogenase and aspartate aminotransferase.[18,19] In our population no interindividual differences for these predictors were seen. Treatment related risk factor for neutropenia event are prior chemotherapy and the intensity of chemotherapy.[14] Only one patient received prior chemotherapy (FEC; 5-Fluorouracil, epirubicin, cyclophosphamide), and almost all patients received TAC (docetaxel, adriamycin, cyclophosphamide) chemotherapy. The greater the myelosuppressive potential of chemotherapy the lower the ANC nadir,[20] which in turn could enhance intrinsic hematopoietic growth factor production and subsequently protect against chemotherapy-induced neutropenia. Little evidence regarding this subject is published. One study demonstrated that in vivo the chemotherapy increases the GM-CSF (granulocyte-macrophage colony stimulating factor), hematopoietic growth factor.[21] So, if this is true we would have expected, after excluding all possible confounders, chemotherapy itself would increase the blood cell counts in the next cycle. However, no such observation was seen.

A limitation of our study is the small number of eligible patients for this analysis, partly due to the relatively high percentage of missing values. This exploratory analysis will form the basis for further research, to draw firm conclusions.

In conclusion, we analysed in an exploratory way chemotherapy-induced hematologic toxicity after exclusion of possible risk factors of neutropenia. Apart from a protective effect of G-CSF for the cycle where G-CSF was given in, myelotoxicity in the consequent cycle seemed not to improve by prior chemotherapy or prior G-CSF.

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Chapter 6

Ultrasound is at least as good as magnetic resonance imaging in predicting tumour size post-neoadjuvant chemotherapy in breast cancer

Birgit EPJ Vriens, Bart de Vries, Marc BI Lobbes, Saskia M van Gastel,
Franchette WPJ van den Berkmortel, Tineke J Smilde,
Laurence JC van Warmerdam, Maaike de Boer, Dick Johan van Spronsen,
Marjolein L Smidt, Petronella GM Peer, Maureen J Aarts, Vivianne CG Tjan-Heijnen,
on behalf of the INTENS study group

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Abstract

Background

The aim of this study was to evaluate the accuracy of clinical imaging of the primary breast tumour post-neoadjuvant chemotherapy (NAC) related to the post-neoadjuvant histological tumour size (gold standard) and whether this varies with breast cancer subtype. In this study results of both magnetic resonance imaging (MRI) and ultrasound (US) were reported.

Methods

Patients with invasive breast cancer were enrolled in the INTENS study between 2006 and 2009. We included 182 patients of whom data were available for post-NAC MRI (n=155), US (n=123) and histopathological tumour size.

Results

MRI estimated residual tumour size with <10 mm discordance in 54% of patients, overestimated size in 28% and underestimated size in 18% of patients. With US this was 63%, 20% and 17%, respectively. The negative predictive value in hormone receptor (HR) positive tumours for both MRI and US was low, 26% and 33%, respectively. The median deviation in clinical tumour size as percentage of pathological tumour was 63% (P_{25} =26, P_{75}=100) and 49% (P_{25} =22, P_{75}=100) for MRI and US, respectively (P=0.06).

Conclusions

In this study, US was at least as good as breast MRI in providing information on residual tumour size post-neoadjuvant chemotherapy. However, both modalities suffered from a substantial percentage of over- and underestimation of tumour size and in addition both showed a low negative predictive value of pathologic complete remission (Gov nr: NCT00314977).

Introduction

Neoadjuvant chemotherapy in breast cancer patients provides the opportunity to monitor treatment effects in vivo and has the potential to downstage the primary tumour which may facilitate breast conservative surgery.

Internationally, it is agreed to monitor clinical tumour response to chemotherapy by the so-called Response Evaluation Criteria in Solid Tumours (RECIST).[1] In case of tumour progression while on chemotherapy, treatment will be changed to immediate surgery or to another systemic treatment option. If the tumour is considered irresectable, locoregional radiotherapy may be an alternative treatment option. In case of stable disease, most guidelines recommend to continue systemic treatment, but results from a recent study suggest that a switch to a non-cross resistant regime may in this situation improve long-term outcome.[2] Hence, in vivo response measurement influences treatment decisions while on neoadjuvant chemotherapy.

One of the goals of clinical therapy monitoring is, to identify any residual tumour in order to plan the most appropriate surgical technique. Importantly, the rate of successfully achieving a free resection margin is a quality indicator of the multidisciplinary breast cancer team according to the European Society of Breast Cancer Specialists (EUSOMA).[3]

In studies on *primary breast surgery* magnetic resonance imaging (MRI) was shown to be most reliable in assessing histological tumour size.[4-5] Ultrasound (US) underestimated tumour size significantly by 18%, whereas MRI did not show any significant deviation from histological tumour size.[4] However, in a review including more recent studies MRI accuracy was reported relatively lower.[6] Also in the *neoadjuvant* setting, conflicting findings are reported. Some studies suggest that breast MRI is more reliable than any of the conventional methods in the assessment of residual tumour tissue while other studies suggest that there is no difference between MRI and US.[7-10] Only few studies studied the impact of histological breast cancer subtype and other tumour characteristics on imaging accuracy.

In the present prospective study, we aimed to compare the accuracy of clinical breast tumour size measurement post-neoadjuvant chemotherapy by MRI or by US with the post-neoadjuvant pathologic tumour size as gold standard. We also aimed to study whether this varied with histological breast cancer subtype. Furthermore, we aimed to determine the ability of a 'negative' MRI to predict pathologic complete remission (pCR) at surgery, per histological breast cancer subtype. The current imaging study was part of a clinical randomised phase III study in which effectiveness of two neoadjuvant chemotherapy regimens was compared.[11]

Patients and methods

The Clinical Study

The results of the clinical study, the INTENS study, have been reported before.[11] In short, the INTENS study was a multicentre, open-label, phase III study in patients with newly diagnosed breast cancer (Gov no. NCT00314977). Patients were randomly assigned to neoadjuvant chemotherapy consisting of four cycles of three-weekly doxorubicin (A) and cyclophosphamide (C) followed by four cycles of docetaxel (T) or six cycles of TAC every three weeks. The primary endpoint was pCR rate, defined as no invasive tumour present in the breast. AC-T resulted in pCR in 21% and TAC in 16% of patients with an odds ratio of 1.44 (95% confidence interval (CI) 0·67-3·10). Patients with a triple-negative tumour (estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) negative) had the highest pathologic tumour response rate (38%).

Breast Imaging Study

For the present study, we included patients from the INTENS trial (n=201) of whom data on tumour size were available measured by MRI and/or US post-neoadjuvant chemotherapy and who underwent surgery to determine pathologic response (Figure 6.1).

Radiologic tumour size measurements were based on the largest diameter of the invasive tumour examined by study centre radiologists. Dynamic contrast-enhanced breast MRI was performed on scanners having >1.5 T field strength with a dedicated breast coil. Although MRI systems and sequence protocols might vary slightly between the different study centres, they all comply with the guidelines of the European Society of Breast Imaging (EUSOBI).[12-13] Consequently, the sequence protocols consisted of a T2w sequence with or without fat suppression, combined with a dynamic, contrast-enhanced T1w sequence series. Radiologic tumour size measurements were based on the maximum size of the invasive tumour, measured on the dynamic T1w sequences using digital calipers on the imaging workstation. Tumour size definitions were based on the region of abnormal enhancement. For US high-frequency breast US transducers were used and digital calipers to measure the tumour size. The clinical tumour response was rated using RECIST 1.0.[1] Although no specific training was given to standardize measurements, all radiologists performing the measurements were experienced breast radiologists.

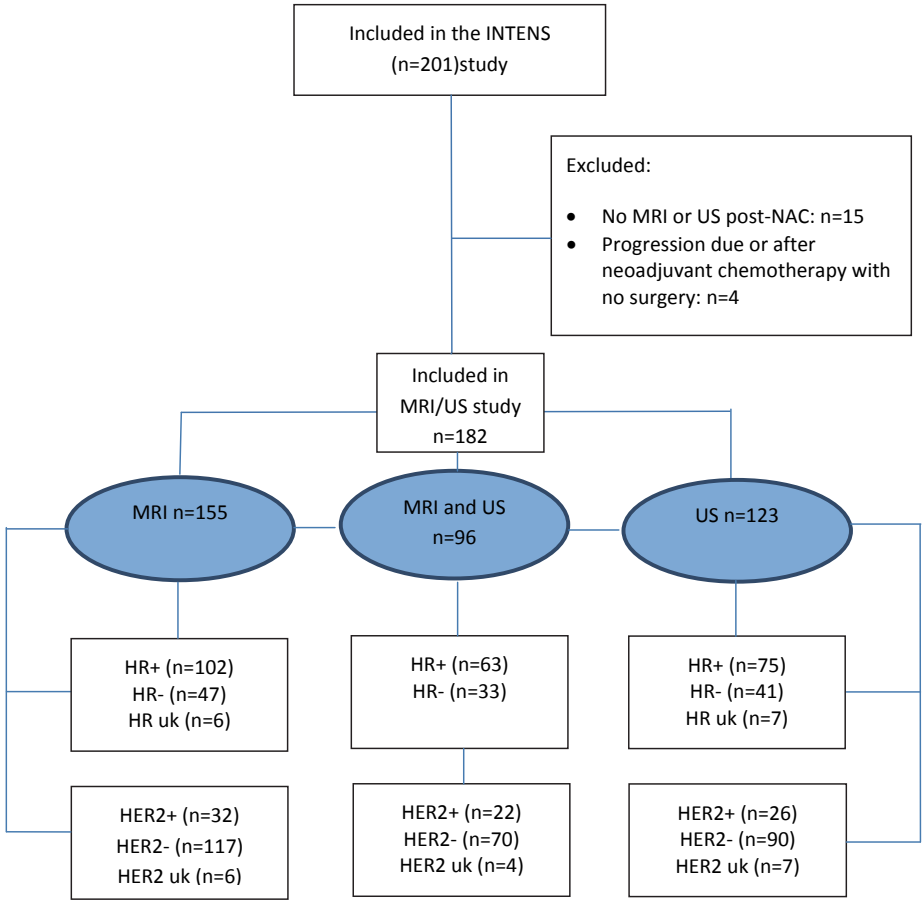

Figure 6.1 Consort diagram

Histopathology analysis

Prior to neoadjuvant chemotherapy, biopsies of the primary tumour (14 gauge) were taken for histological analysis. Tumours were classified in four subgroups according to their receptor status.

Pathologic breast tumour response was assessed by the Miller and Payne grading.[14] Grade 5 pathologic response (i.e., pCR) was scored if there were no malignant cells identifiable in sections from the site of the tumour, the presence of ductal carcinoma in situ (DCIS) was accepted. Resection specimens were centrally reviewed (BdV). Further, tumour grade (Bloom and Richardson), architecture, atypia, mitosis and stromal characteristics (inflammation, desmoplasia, fibro-elastosis) was re-assessed in the baseline biopsy.

Statistical analysis

The primary goal was determination of the accuracy of clinical imaging of the primary breast tumour post-neoadjuvant chemotherapy, defined as agreement in size (<10mm difference) per hormone receptor (HR)/HER2 subtype between breast MRI and pathologic breast tumour size and between breast US and pathologic tumour size. In addition, we assessed the association between baseline pathologic tumour characteristics and the degree of over- or underestimation of residual tumour by MRI or US.

Moreover, we determined the positive predictive value (PPV) and negative predictive value (NPV) by MRI or US post-neoadjuvant chemotherapy per HR subtype. The PPV is defined as the probability that tumour is actually present if the imaging reports that it is present. NPV is defined as the probability that tumour is absent (pCR) if the imaging reports a clinical complete response (cCR). Apart from pCR we also assessed PPV and NPV for the assessment of residual tumour of respectively ≤10, 20 or 30 mm, as we consider this important for surgical decision-making. For the sake of comparability, we now defined "true-negative (TN)" as a tumour size by imaging of $\underline{\leq}$10, 20, 30 mm, respectively and a postoperative equivalent pathology tumour size.

In non-pCR patients who underwent clinical tumour size assessment by both MRI and US, the Wilcoxon signed rank test was applied on the difference between the paired deviations from pathologic tumour size for MRI- imaging and US-imaging. To avoid any assumption on the statistical distribution of this parameter, we chose to perform a non-parametric test. This test incorporates the magnitude of the differences by ranking and therefore the test result will not be affected by the presence of outliers. As the median absolute difference in breast tumour size by MRI or US and pathologic tumour size was expressed as percentage of the pathologic tumour size, patients with pCR were excluded as for these the denominator is zero. A *P* value ≤0.05 was considered statistically significant.

Results

Patients

From February 2006 through April 2009, a total of 201 patients from 21 hospitals in the Netherlands were enrolled in the phase III INTENS study, in which they were randomised between AC-T and TAC neoadjuvant chemotherapy.[11] We included 182 patients, of these 155 had undergone MRI and 123 US for clinical tumour response assessment (Figure 6.1, Tables 6.1A and 6.1B). 96 patients had clinical tumour response evaluation by both MRI and US.

Table 6.1A Clinical breast tumour size on MRI versus pathologic tumour size (PA) post-neoadjuvant chemotherapy, related to tumour characteristics in the biopsy material.

	N	Overestimation MRI versus PA>10 mm N (%)	Difference |MRI-PA| ≤10 mm N (%)	Underestimation MRI versus PA >10 mm N (%)
All	155	44 (28)	83 (54)	28 (18)
Receptor status				
HR+/HER2-	82	20 (24)	42 (51)	20 (24)
HR+/HER2+	20	9 (45)	9 (45)	2 (10)
HR-/HER2-	35	10 (29)	20 (57)	5 (14)
HR-/HER2+	12	4 (33)	7 (58)	1 (8)
Unknown	6	1	5	0
Tumour Grade (B&R)				
Grade I and II	84	24 (29)	41 (49)	19 (23)
Grade III	51	17 (33)	31 (61)	3 (6)
Unknown	20	3	11	6
Mitoses				
1	58	17 (29)	29 (50)	12 (21)
2	46	11 (24)	26 (57)	9 (20)
3	31	13 (42)	17 (55)	1 (3)
Unknown	20	3	11	6
Stromal characteristics				
Inflammation				
0	26	5 (19)	15 (58)	6 (23)
1	70	21 (30)	39 (56)	10 (14)
2	34	15 (44)	14 (41)	5 (15)
Unknown	25	3	15	7
Apoptosis				
0	43	9 (21)	23 (53)	11 (26)
1	42	17 (40)	22 (52)	3 (7)
2	11	2 (18)	9 (82)	0
Unknown	59	16	29	14
Desmoplasia				
0	10	1 (10)	8 (80)	1 (10)
1	102	29 (28)	57 (56)	16 (16)
2	17	10 (59)	3 (18)	4 (24)
Unknown	26	4	15	7

HR Hormone receptor, *HER2* Human epidermal growth factor receptor 2, *MRI* magnetic resonance imaging, *PA* pathologic tumour size.

Table 6.1B Clinical breast tumour size on US versus pathologic tumour size (PA) post-neoadjuvant chemotherapy, related to tumour characteristics in the biopsy material.

	N	Overestimation US versus PA >10 mm N (%)	Difference |US-PA| ≤10 mm N (%)	Underestimation US versus PA >10 mm N (%)
All	123	25 (20)	77 (63)	21 (17)
Receptor status				
HR+/HER2-	59	11 (19)	33 (56)	15 (25)
HR+/HER2+	16	3 (19)	12 (75)	1 (6)
HR-/HER2-	31	11 (35)	17 (55)	3 (10)
HR-/HER2+	10	0 (0)	9 (90)	1 (10)
Unknown	7	0	6	1
Tumour Grade (B&R)				
Grade I and II	69	11 (16)	42 (61)	16 (23)
Grade III	37	10 (27)	26 (70)	1 (3)
Unknown	17	4	9	4
Mitoses				
1	48	7 (15)	30 (63)	11 (23)
2	27	4 (15)	18 (67)	5 (19)
3	31	10 (32)	20 (65)	1 (3)
Unknown	17	4	9	4
Stromal characteristics				
Inflammation				
0	25	6 (24)	14 (56)	5 (20)
1	54	6 (11)	39 (72)	9 (17)
2	25	9 (36)	13 (52)	3 (32)
Unknown	19	4	11	4
Apoptosis				
0	33	5 (15)	19 (58)	9 (27)
1	28	6 (21)	18 (64)	4 (14)
2	11	3 (27)	8 (73)	0 (0)
Unknown	51	11	32	8
Desmoplasia				
0	11	3 (27)	7 (64)	1 (9)
1	79	16 (20)	50 (63)	13 (16)
2	14	2 (14)	9 (64)	3 (21)
Unknown	19	4	11	4

HR Hormone receptor, *HER2* Human epidermal growth factor receptor 2, *US* ultrasound, *PA* pathologic tumour size.

Accuracy of clinical imaging and over- and underestimation of residual tumour size

The median breast tumour size on MRI was 40 mm (range 12-120 mm) in diameter before and 18 mm (range 0-110 mm) post-neoadjuvant chemotherapy, with a median pathologic tumour size in the resection specimen of 15 mm (range 0-90 mm). For US, these numbers were 30 (range 7-100 mm), 18 (range 0-100 mm) and 18 mm (range 0-80 mm), respectively.

MRI estimated residual tumour size with ≤10 mm discordance in 54% of the patients (Table 6.1A). MRI overestimated clinical tumour size by >10 mm especially in patients

with HER2 positive disease (41%) triple negative disease (29%), grade III tumours (33%), grade III mitosis (42%), grade I/II stromal inflammation (35%), apoptosis (36%), or desmoplasia (33%).
US estimated the residual tumour size correctly in 63% of patients (Table 6.1B). US overestimated clinical tumour size by >10 mm especially in patients with triple negative disease (35%), grade III mitosis (32%) and grade II stromal inflammation (36%).

Predicting values for presence of residual tumour or pCR

With MRI the PPV of predicting the presence of residual tumour in the breast was 92% in HR positive tumours and 80% in HR negative tumours (Table 6.2A). However, the negative predictive value was extremely low for HR positive tumours, 26%, compared to 58% for HR negative tumours. Comparably, the PPV of US was 92% in HR positive tumours and 75% in HR negative tumours. Also with US the NPV was extremely low for HR positive tumours, 33%, compared to 78% for HR negative tumours (Table 6.2B). HER2 status did not clearly impact accuracy rates (data not shown).
When relating clinical and pathological tumour size post-neoadjuvant chemotherapy with different definitions of TN findings (pCR, 0-10, 0-20, 0-30 mm), the negative predictive value increased, whereas the PPV decreased with increasing tumour range for both MRI and US. (Table 6.2A and 6.2B).

Table 6.2A Post-chemotherapy clinical breast tumour size (MRI) versus pathologic tumour size. Relation between presence and absence of residual tumour (pCR) and residual tumour size of ≤10, 20 and 30 mm.

	Total N	pCR N	TN N	FN N	TP N	FP N	PPV %	NPV %
MRI								
HR+	102	13	7	20	69	6	92	26
HR-	47	14	7	5	28	7	80	58
	Total N	0-10mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
MRI								
HR+	102	36	20	17	49	16	75	54
HR-	47	22	13	4	21	9	70	76
	Total N	0-20mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
MRI								
HR+	102	68	48	15	19	20	49	76
HR-	47	27	21	7	13	6	68	75
	Total N	0-30mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
MRI								
HR+	102	87	73	11	4	14	22	87
HR-	47	36	31	2	9	4	69	94

Apart from pCR, residual tumour of respectively ≤10, 20 or 30 mm was assessed. *HR* hormone receptor status, *pCR* pathologic complete remission, *MRI* magnetic resonance imaging, *TN* true negative, *FN* false negative, *TP* true positive, *FP* false positive, *PPV* positive predictive value=TP/(TP+FP); *NPV* negative predictive value=TN/(TN + FN).

Table 6.2B Post-chemotherapy clinical breast tumour size (US) versus pathologic tumour size. Relation between presence and absence of residual tumour (pCR) and residual tumour size of ≤10, 20 and 30 mm.

	Total N	pCR N	TN N	FN N	TP N	FP N	PPV %	NPV %
US								
HR+	75	9	4	8	58	5	92	33
HR-	41	15	7	2	24	8	75	78
	Total N	0-10mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
US								
HR+	75	24	14	10	41	10	80	58
HR-	41	26	16	2	13	10	57	89
	Total N	0-20mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
US								
HR+	75	49	37	19	7	12	37	66
HR-	41	29	23	5	7	6	54	82
	Total N	0-30mm N	TN N	FN N	TP N	FP N	PPV %	NPV %
US								
HR+	75	66	62	9	0	4	0	87
HR-	41	34	32	2	4	3	57	94

Apart from pCR, residual tumour of respectively ≤10, 20 or 30 mm was assessed. *HR* hormone receptor status, *pCR* pathologic complete remission, *US* ultrasound, *TN* true negative, *FN* false negative, *TP* true positive, *FP* false positive, *PPV* positive predictive value=TP/(TP+FP); *NPV* negative predictive value=TN/(TN + FN)

Size discrepancies MRI and US and final pathological size

Over- and underestimation related to the increasing magnitude of the post-chemotherapy histopathological tumour size were present in both MRI and US (Figure 6.2).

Because of increasing deviation of imaging tumour sizes with increasing histologic tumour size (Figure 6.2), the deviations were expressed in relative terms (Table 6.3).

In patients that underwent both MRI and US (n=96), non-pCR (n=76), the median absolute difference in clinical and pathologic tumour size as percentage of post-chemotherapy pathologic tumour size (ypT size) was 63% (P_{25} =26, P_{75}=100) for MRI and 49% (P_{25} =22, P_{75}=100) (p=0.06) for US. Similar results were seen in patients with HR positive as in those with HR negative tumours (Table 6.3). HER2 status did not clearly impact these results(results not shown).

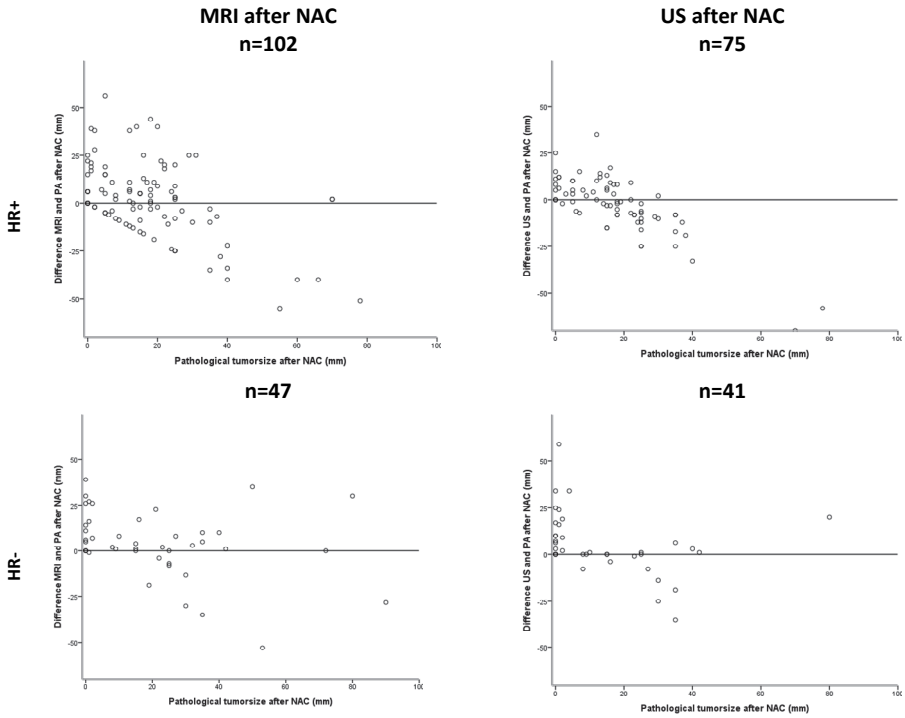

Figure 6.2 Difference between tumour size by MRI or US and pathological tumour size (mm), plotted against the pathological tumour size post-neoadjuvant chemotherapy (NAC).

Table 6.3 Absolute difference of clinical tumour size on MRI or US and pathological tumour size (mm) post-neoadjuvant chemotherapy as percentage of pathological tumour size, related to the hormone receptor (HR) status in the biopsy material.

		MRI	US	p-Value
	N(%)	Median|MRI-PA|/PA % (P_{25}-P_{75})	Median|US-PA|/PA % (P_{25}-P_{75})	
All*	76 (100)	63% (26-100)	49% (22-100)	0.06
HR+	54(71)	82% (32-100)	50% (27-100)	0.11
HR-	22(29)	30% (16-101)	28% (4-100)	0.25

*Patients were included who had post-chemotherapy both MRI and US and residual tumour (see Methods).
HR hormone receptor status, *MRI* magnetic resonance imaging, *US* ultrasound.

Discussion

We compared the clinical tumour size as assessed by MRI and US post-neoadjuvant chemotherapy with the postoperative pathologic tumour size. Breast MRI post-

neoadjuvant chemotherapy overestimated tumour size in a significant proportion of patients (28%) by >10 mm, especially in patients with more aggressive tumour characteristics, whereas MRI underestimated tumour size by >10 mm in 18% of patients, especially in those with HR positive tumours. In comparison, US estimated pathologic tumour size correctly in 63% of patients, overestimated tumour size in 20% and underestimated tumour size in 17% of patients by >10 mm.

Of relevance, when directly comparing the absolute difference in clinical tumour size by MRI or US and pathological tumour as percentage of pathological tumour size in the patients who underwent both imaging modalities, the median size deviation was 63% and 49% (p=0.06) for MRI and US, respectively.

Only a few small studies have been reported comparing tumour diameter on MRI and US post-neoadjuvant chemotherapy with pathological results as the gold standard.[10,15,16] In two studies, MRI was slightly superior to US, whereas in one study accuracy was comparable. Pengel et al. showed that the combined use of MRI and positron-emission tomography/ computed tomography had the potential to improve the ability to predict final tumour response at pathology during neoadjuvant chemotherapy in comparison with one of the imaging modalities separately.[17]

In our study, the NPV of pCR was 26% with MRI in patients with HR positive disease, which implies that in 74% of patients with no suspicious areas on MRI post-neoadjuvant chemotherapy still residual tumour was present. Actually, in these (74%) patients postoperative median pathologic tumour size was 14 mm (range 2-55 mm). A comparable low NPV of pCR of 27% was seen in another study that studied MRI accuracy post-chemotherapy in patients with HR positive disease.[18] With US the NPV of pCR in HR positive disease was also low (33%). The surgical resection margins may, therefore, be at risk of tumour involvement.

In the entire INTENS study population, which was a multicentre study in 21 centres of the Netherlands, in 5 of 63 (8%) patients who initially underwent breast conserving surgery the resection margin was not free for invasive cancer.[11]

For patients diagnosed with breast cancer in the period 2008-2009 in the Netherlands, the National Breast Cancer Registry (NBCA) reported 9.1% (95% CI 8.4–9.8%) invasive breast cancer with a positive surgical margin status after first lumpectomy.[19] In 2013 the NBCA reported a lower rate of 5% for margin involvement in invasive breast cancer for patients who underwent primary lumpectomy and 7.6% for patients who had a lumpectomy post-neoadjuvant chemotherapy.[20] Hence, the margin involvement rate in the INTENS study is in agreement with the rates seen in daily practice, whereby margin involvement rates have progressively declined during recent years in the Netherlands. In a retrospective analysis no difference in margins status between post-neoadjuvant chemotherapy and primary surgery was reported, although with much higher rates of margin involvement than in our study of 21% and 18%, respectively (p=0.52, n=478).[21] Another definition of margin involvement, without imaging post-neoadjuvant chemotherapy may explain the different rates.

In our study, in which we included patients in the years 2006-2009, it was recommended to continue the neoadjuvant chemotherapy in the presence of stable disease after three cycles of TAC chemotherapy. In patients randomised to AC-T, treatment was by protocol switched halfway, irrespective of clinical response. Recently, von Minckwitz et al showed that response-guided neoadjuvant chemotherapy (switch in case of stable disease) resulted in an improved disease-free survival.[2] However, as our study shows that post-neoadjuvant chemotherapy imaging does not have a high accuracy, how should we then interpret clinical tumour response on systemic therapy, and how should we use RECIST criteria? In our study, of the 34 patients with a clinical complete response only 12 patients (35%) had a pCR in the breast. From the patients without residual disease in the breast (ypT0, n=31) only 39% also had a clinical complete response by imaging. In our study MRI did also not reveal differences in clinical complete response rates between HR tumour subtypes (26%) in sharp contrast to pathologic examination, with relatively high pCR rates (pathologically no evidence of disease) in HR negative tumours (30%) and low pCR rates in HR positive tumours (13%), see Table 6.3A. Apparently, other markers of response (biomarkers) and novel imaging techniques to identify absence of response in a reliable manner are urgently needed. Only in this way the patients may fully benefit from neoadjuvant chemotherapy use as opposed to 'blind' adjuvant chemotherapy.

pCR was the primary endpoint of our clinical study and, although pCR is associated with overall survival, it should be noted that debate is still ongoing on the clinical relevance of improving pCR in relation to improving outcome. Von Minckwitz et al. reported that pCR is a suitable surrogate end point for patients with HER2-positive, triple negative or luminal B/HER2-negative tumours, but that the association between improved pCR and survival outcome is less pronounced for patients with luminal-A-like tumour.[22] A recent pooled analysis of the CTNeoBC could not validate pCR as a surrogate endpoint for improved event free and overall survival.[23]. In 2013, the FDA provided accelerated approval to pertuzumab as part of neoadjuvant treatment of patients with HER2-positive breast cancer. The pCR rates of the underlying clinical study was defined by the FDA-preferred definition, i.e., absence of invasive cancer in the breast and lymph nodes (ypT0/is ypN0).[24] This approval was based on the observed increasement in pCR rates in the neoadjuvant studies, combined with the observed improved survival in the metastatic CLEOPATRA trial.[25-27] Now that the ALTTO study with adjuvant double HER2-targeting (trastuzumab/lapatinib) has not resulted in improved survival despite higher pCR rates in the corresponding neoadjuvant Neo-ALTTO study, discussion on the value of pCR as endpoint is re-opened.[28]

Some studies tried to identify factors to predict MRI accuracy post-neoadjuvant chemotherapy. Chen et al. showed in a multivariate analysis that tumour type (ductal versus lobular), tumour morphology (mass lesion versus non-mass like), and biomarkers (HR and HER2 status) were all independent predictors of MRI accuracy.[29]

In our study we found that triple negative disease, HER2 positive disease, high histological grade, stromal inflammation and apoptosis were related to overestimation of tumour size by MRI and US. Interestingly, increasing evidence suggests that tumour infiltrating lymphocytes (TILs) are prognostic and predictive for breast cancer outcome, specifically in triple negative and HER2 positive breast cancer patients.[30] Chemotherapy may trigger an immune response by TILs, which contributes to treatment response. It is imaginable that this response post-neoadjuvant chemotherapy results in an enhanced signal related to the presence of TIL's and not to the tumour. This will lead to overestimation of residual disease and reduces accuracy of clinical tumour size assessment. This may also explain why MRI may be more reliable in the primary surgery setting as compared to the post-neoadjuvant chemotherapy setting.[4-5] In absence of TILs confounding factors in overestimating tumour size might be: reactive inflammation caused by tumour response and healing, surrounding sclerosis and necrosis, multiple scattered lesions and presence of accompanying ductal carcinoma in situ.[7]

A high correlation for imaging and histopathological size does not automatically imply that there is good agreement between the two methods.[7] For this reason it seems inappropriate to use correlation efficients for comparison of residual tumour size with MRI or US. Therefore, we choose to analyse agreement between these measurements by plotting the difference between the imaging and the histological results against the pathological tumour size (gold standard). This is an adaption of the Bland-Altman plot where the mean of two diagnostic tests is presented at the horizontal axis instead of the gold standard.[31-32]

Various expert panels recommend to repeat receptor status testing in circumstances where the pathologic findings of the resection specimen may yield a different result from the biopsy that would change treatment.[33-34] We based the phenotype of disease on the biopsy HR status, which may be considered a limitation of our study, although in absolute terms the numbers are small with conversion of HR status. In 43% of patients with HR negative tumour on the baseline biopsy and residual tumour present after neoadjuvant chemotherapy (n=18) the receptor status was repeated on the resection specimen, none of these resulting in a positive HR status. In the remaining n=24 patients HR status was not repeated. Although we performed central pathology review for all included patients, we have not performed a central MRI review, which we consider a limitation of our study.

In conclusion, imaging post-neoadjuvant chemotherapy showed a low negative predictive value especially in HR positive tumours. MRI and US showed comparable (moderate) agreement with post-neoadjuvant chemotherapy pathological tumour size. Initiating studies of new imaging approaches and response-predicting biomarkers, in the setting of studying novel therapies for "non-ideal" responders, is most important. Now, we must move forward, beyond the standard of US and MRI in this age.

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Chapter 7

Accuracy of sentinel node biopsy after neoadjuvant chemotherapy in breast cancer patients: a systematic review

Carolien HM van Deurzen, Birgit EPJ Vriens, Vivianne CG Tjan-Heijnen, Elsken van der Wall, Mirjam Albregts, Richard van Hilligersberg, Evelyn M Monninkhof, Paul J van Diest

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Abstract

Background

As neoadjuvant chemotherapy (NAC) is increasingly used to downstage patients with breast cancer, the timing of the sentinel node (SN) biopsy has become an important issue. This review was conducted to determine the accuracy of SN biopsy following NAC.

Methods

We searched Medline, Embase and Cochrane databases from 1993 through February 2009 for studies on patients with invasive breast cancer who underwent SN biopsy after NAC followed by an axillary lymph node dissection (ALND).

Results

Of 574 eligible studies, 27 were included in this review with a total study population of 2148 patients. The pooled SN identification rate was 90.9% (95% confidence interval (CI)=88.0% to 93.1%) and the false-negative rate was 10.5% (95% CI=8.1% to 13.6%). Negative predictive value and accuracy after NAC were 89.0% (95% CI=85.1% to 92.1%) and 94.4% (95% CI=92.6% to 95.8%), respectively. The reported SN success rates were heterogeneous and several variables were reported to be associated with decreased SN accuracy, i.e. initially positive clinical nodal status.

Conclusions

There is a potential role for SN biopsy following NAC which could be considered on an individual basis. However, there is insufficient evidence to recommend this as a standard procedure. Further research with subgroup analysis using variables reported to be associated with decreased SN accuracy is required in order to clearly define its value in the subgroups of breast cancer patients.

Introduction

Lymph node status, even after neoadjuvant chemotherapy (NAC), is a strong predictor for disease-free and overall survival in breast cancer patients.[1-3] A sentinel node (SN) biopsy is an accurate method to assess nodal status and has now replaced traditional axillary lymph node dissection (ALND) as an initial staging procedure in early-stage, clinically node-negative breast cancer patients.[4,5] Several studies indicated that SN biopsy is also feasible for patients with large primary breast tumours,[6-8] provided there is no clinical nodal involvement.[9]

NAC, initially introduced to downstage locally advanced breast cancer to facilitate surgery, results in an improved disease-free and overall survival, which is comparable with the effect of adjuvant chemotherapy.[10-13] More recently, the indication for NAC has been extended to selected patients with earlier stage disease to allow breast-conserving surgery.[14,15] Another potential advantage of a neoadjuvant approach is the opportunity to observe chemosensitivity in situ, providing prognostic information and the ability to identify effective novel therapies.[16]

Following NAC, nodal staging was traditionally performed by an ALND at the time of breast surgery, which is associated with substantial morbidity.[17,18] Therefore, a less aggressive approach to the axilla is desirable. In fact, this raises not only the question whether these patients could be staged by SN biopsy, but also the question of what the optimal timing is for this procedure with respect to the NAC.

Performing an SN biopsy before NAC, on the one hand, assures accurate assessment of initial nodal status, avoiding the possible negative effects of lymphatic scarring or uneven nodal tumour response. On the other hand, performing an SN biopsy after NAC, could be an attractive strategy as NAC may downstage nodal status in a number of patients (20-40%).[14,19] Before such a strategy can be recommended as a routine procedure, validation of the safety and predictive value of SN biopsy following NAC is required.

Numerous, generally retrospective, small and single-institution studies assessed the feasibility of SN biopsy after NAC, with varying conclusions. This systematic review was conducted to give an overview of these studies and provide recommendations regarding the role of SN biopsy following NAC.

Methods

Literature search strategy

The electronic databases of Medline, Embase and Cochrane were searched from 1993 through February 2009 using free text and controlled terms for breast cancer, SN and NAC. The year 1993 was selected because this was the year of the first publication on the SN. Articles published in English, German, French or Dutch were considered. Two reviewers (C.H.M. van Deurzen and B.E.PJ. Vriens) independently evaluated titles and

abstracts of the identified papers. Potentially relevant articles were retrieved to review the full text.

Study inclusion criteria

To be included in this review, studies had to fulfil the following criteria. First, patients had received NAC for invasive breast carcinoma. Second, the patients underwent an SN biopsy after NAC, which was followed by an ALND. The patients receiving neoadjuvant endocrine therapy only were excluded.

Data extraction

Data extraction was carried out independently by the two reviewers who abstracted data on entry criteria, size, clinicopathologic characteristics, SN biopsy technique, SN success rates, SN and non-SN status. Any disagreement was resolved by consensus. In some instances, corresponding authors were contacted for additional information.
SN accuracy parameters were recalculated according to standard definitions in order to facilitate comparison across studies. The identification rate was defined as the number of patients who underwent a successful SN biopsy divided by the total number of patients in whom an SN biopsy was attempted. The result from each successfully identified SN was categorised as true-positive, true-negative or false-negative, taking the outcome of the complete ALND as reference standard. A true-negative SN was defined as a negative SN and a negative ALND, a false-negative as a negative SN with a positive lymph node in the ALND and a true-positive as a positive SN with or without a positive ALND. Based on these definitions, it was assumed that there were no false-positive cases.
The calculated SN accuracy parameters included sensitivity (true positive/(true positive + false negative)), false-negative rate (false negative/(false negative + true positive)) and negative predictive value (true negative/(true negative + false negative)). Accuracy was computed as the sum of all true-positive and true-negative patients, divided by the number of patients with a successfully identified SN.

Statistical analysis

A random effects model with an exact likelihood approach was used to calculate pooled SN accuracy parameters and 95% confidence intervals (CIs).[20] The extent to which clinical nodal status explained between-study heterogeneity was explored by use of meta-regression analysis using the same model.[21] The variation in accuracy parameters in the individual studies was displayed graphically using forest plots. The 95% CIs for the individual studies were calculated by use of the Rothman spreadsheet.[22] Systematic differences between small and large studies were assessed by the use of a funnel plot. Two sided p-values <0.05 were considered significant. Statistical analyses were performed using SAS version 9.1.

Results

The initial electronic search identified 574 potential relevant articles of which we screened the title and abstract. After screening, the full texts of 66 articles were obtained. After full-text review and exclusion of overlapping series, 27 articles that met the inclusion criteria of this review remained for data extraction, including single- (N=23) and multicentre (N=4) series. The total study population comprised 2148 patients. The main characteristics and results of these studies are listed in Table 7.1.

Table 7.1 Overview of breast cancer studies reporting on SN accuracy following neoadjuvant chemotherapy.

First author	N	cT	cN	IR	FNR	Sens	NPV	Accuracy	SN+ only	SN and non-SN+ §
				%	%	%	%	%	%	%
Nason[23]	15	2-4	0-1	87	33	67	57	77	46	100
Cohen* [24]	38	2-3	0-1	82	17	83	81	90	48	M
Fernandez[25]	40	1-4	0-1	85	20	80	78	88	47	75
Tafra[26]	29	1-2	0	93	0	100	100	100	56	M
Brady[27]	14	1-4	0-1	93	0	100	100	100	77	40
Stearns[28]	34	3-4	0-1	85	14	86	73	90	62	72
Schwartz[29]	21	1-3	0-1	100	9	91	91	95	48	30
Balch[30]	32	2-4	0-1	97	5	95	92	97	58	44
Piato‡ [31]	42	1-2	0	98	17	83	88	93	37	100
Vigario‡ [32]	37	1-2	0	97	39	61	72	81	M	M
Shimazu[33]	47	2-4	0-1	94	12	88	73	91	66	69
Lang[34]	53	1/2-4	0-1	94	4	96	96	98	46	M
Patel# [35]	42	2-4	0-1	95	0	100	100	100	48	M
Shen* [36]	69	1-4	1	93	25	75	62	82	48	73
Mamounas# [37]	428	1-3	0-1	85	11	89	93	96	36	44
Jones[38]	36	2-4	0-1	81	11	89	85	93	55	63
Tanaka[39]	70	1-3	0-1	90	5	95	96	98	30	58
Yu[40]	127	3	0	91	7	93	90	96	55	63
Tausch[41]	167	1-4	0-1	85	8	92	91	96	49	44
Kinoshita[42]	104	2-4	0-1	93	10	90	93	96	37	56
Lee[43]	219	1-4	1	78	5.6	94	87	96	69	M
Newman[44]	54	1-3	1	98	8	92	85	94	62	M
Yamamoto[45]	20	1-3	0-1	100	14	86	93	95	30	100
Gimbergues[46]	129	1-3	0-1	94	14	86	89	93	40	52
Hino[47]	55	2-3	0-1	71	0	100	100	100	46	72
Papa[48]	31	2-3	0	87	16	84	73	89	59	M
Classe[49]	195	0-3	0-1	90	12	88	95	97	26	43
Total	2148			90.9	10.5	89.5	89	94.4	49.0	61.5

N=number of patients; cT=clinical tumour diameter; cN=clinical nodal status: 0=cN0, 1=clinical nodal involvement (either cN1 or cN2), 0-1=combination of patients with clinical node-positive and negative disease. IR=identification rate; FNR=false-negative rate; NPV=negative predictive value, § percentage of patients with non-SN involvement among patients with a positive SN, M=missing data, ‡ Potential overlap of patients, # * Some overlapping patients

Overall, studies were heterogeneous regarding clinicopathologic characteristics. Patients generally presented with large primary breast tumours (range cT1-4). The upper outer quadrant of the breast was the most common primary tumour location.
SNs were usually identified by using a combination of radiocolloid and blue dye that was injected peritumourally. A combination of anthracycline and cyclophosphamide was the most frequently used chemotherapy. The majority of studies (19/27) included both clinically node-negative and node-positive patients, and the remainder were restricted to clinically node-negative (5/27) or node-positive (3/27) patients.
The overall, pooled SN identification rate was 90.9% (95% CI=88.0% to 93.1%).
The false-negative rate, sensitivity, negative predictive value and accuracy after NAC were 10.5% (95% CI=8.1% to 13.6%), 89.5% (95% CI 86.4 to 91.9%), 89.0% (95% CI=85.1% to 92.1%).and 94.4% (95% CI=92.6% to 95.8%), respectively. Forest plots displayed large interstudy variation in SN accuracy parameters (Figure 7.1).
Overall, the pooled rate of SN involvement was 49% (95% CI=32.0 to 66.2%). Patients with SN involvement had a pooled risk of 61.5% (95% CI=52.7 to 69.6%) for non-SN involvement.

SN after NAC, in studies including cN0 versus cN+ patients only

Meta-regression analysis revealed that clinical nodal status at initial diagnosis, before NAC, did not contribute significantly to between-study heterogeneity regarding SN accuracy parameters. The pooled SN identification rate in studies restricted to clinically node-negative patients (N=5 studies, 266 patients) was 92.7% compared to 88.2% in studies restricted to clinically node-positive (N=3 studies, 342 patients) patients. The pooled negative predictive value was 90.6% in clinically node-negative patients, compared to 87.1% in clinically node-positive patients. The pooled accuracy was comparable for both groups (94.4% and 94.5%, respectively). The effect of clinical tumour size could not be assessed since this was reported heterogeneously in the included studies. A funnel plot did not indicate systematic differences in SN accuracy parameters between larger and smaller studies (data not shown).

Features influencing SN accuracy

A minority of studies performed statistical analysis regarding clinicopathologic features influencing SN accuracy following NAC.
Classe and colleagues[49] (N=195) reported a significantly lower SN identification rate in patients with clinical nodal involvement compared to clinically node-negative patients (P=0.008). Hino and colleagues[47] reported that clinical breast tumour size after NAC, clinical axillary nodal status after NAC and Body Mass Index were significantly associated with SN identification rate (P=0.0003, P=0.048 and P=0.008, respectively).

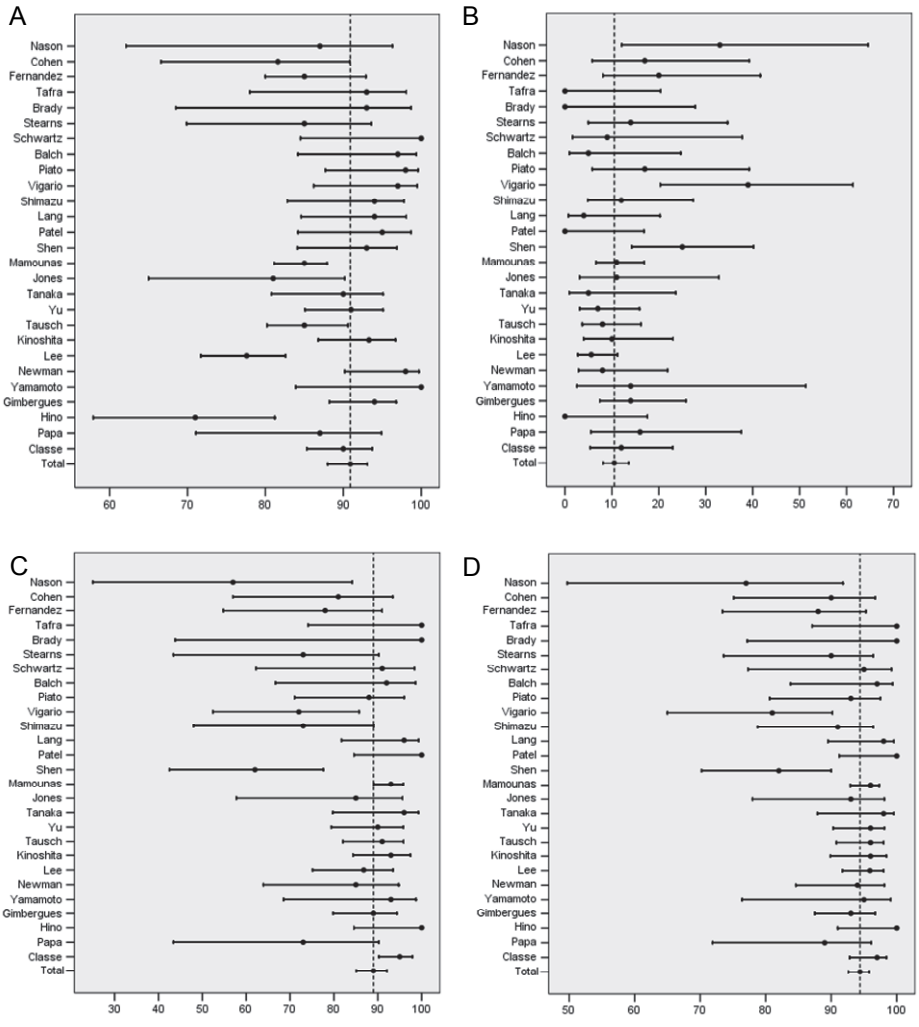

Figure 7.1 Forest plots of SN identification rate (A), false-negative rate (B), negative predictive value (C) and accuracy (D) of an SN biopsy following NAC in breast cancer patients. The width of the horizontal line represents the 95% CI of the individual studies. The 95% CI of the pooled estimate is displayed as a vertical line.

Both Mamounas and colleagues[37] (N=428) and Gimbergues and colleagues[46] (N=129) reported an increased SN false-negative rate with increasing primary tumour size (P=NS and P=0.045, respectively). Gimbergues and colleagues[46] concluded that clinical nodal status before NAC was the main factor influencing the SN false-negative rate after NAC. They reported no false-negative cases in clinically node-negative patients compared to a false-negative rate of 30% in clinically node-positive patients (P=0.003). Tausch and colleagues[41] (N=169) also reported a trend toward a higher false-negative

rate in initial clinically node-positive patients although this was not significant (P=0.39). Mamounas and colleagues[37] (N=428), Kinoshita[42] (N=104) and Classe and colleagues[49] (N=195) on the other hand reported no effects of clinical nodal status on the SN false-negative rate.
Another factor reported to affect a successful SN biopsy is the response to chemotherapy. Tauch and colleagues[41] reported a significantly lower sensitivity among patients with a complete response compared to the group of patients with any other type of response (P=0.048). However, this finding is contradicted by Mamounas and colleagues[37] who reported a trend towards a lower sensitivity in patients with a poor tumour response.

Discussion

This systematic review was conducted to give an overview of the current literature regarding the accuracy of an SN biopsy in breast cancer patients following NAC. We calculated a pooled SN identification rate and false-negative rate of 90.9% and 10.5%, respectively. These rates do not differ substantially from prior multicentre studies evaluating SN success rates without NAC, reporting an identification rate of 88-97% and a false-negative rate of 5-10%.[50-55] However, these rates are largely based on early SN studies and these rates are not generally accepted any more according to current treatment guidelines. The reported pooled false-negative rate of 10.5% in this review is substantially higher than the generally accepted 5% rate without NAC and therefore the SN biopsy following NAC can not be recommended as the standard of care. Besides, several clinicopathologic factors have been reported to be associated with decreased SN accuracy following NAC, including clinical nodal status, primary tumour size, Body Mass Index and response to chemotherapy.
The largest cohort to date, the NSAPB B-27 multicentre randomised trial (N=428)[37], evaluating the sequencing of chemotherapy, reported an identification rate, a false-negative rate and an accuracy of 85%, 11% and 96%, respectively. They concluded that these rates are comparable to those obtained from multicentre studies evaluating SN biopsy following breast cancer diagnosis and suggest that this procedure is feasible following NAC, which is consistent with the results of a meta-analysis by Xing and colleagues.[56] Lee and colleagues[43] reported the largest cohort (N=219) restricted to clinically lymph node-positive patients published to date. They reported an SN identification rate of 78 % after NAC, which was significantly lower (P<0.001) compared to the patients who did not receive NAC. However, the false-negative rate and accuracy did not significantly differ between these two groups. They concluded that an SN biopsy is feasible in patients who reach clinically complete axillary clearance by NAC.
NAC downstages nodal status in a substantial proportion of patients (20-40%),[14,19] which could be an important advantage of nodal staging after NAC compared to nodal

staging before NAC. Other potential advantages of this first approach are avoidance of a delay for NAC and that patients with a negative SN following NAC require only one surgical procedure instead of two. Performing an SN procedure before NAC on the other hand results in two surgical procedures regardless of SN status (one for the SN procedure and one for surgery of the primary tumour). However, Kilbride and colleagues[57] recently reported that knowing nodal status before NAC (by ultrasound with FNA or SN biopsy) provides important prognostic information regarding the risk of recurrence, which may be useful in further treatment planning (i.e. adjuvant irradiation).

Theoretically, NAC could have several negative effects on the accuracy of the SN biopsy. First, both primary tumours and metastatic lymph nodes respond to chemotherapy yielding reactive changes like fibrosis which may affect lymphatic drainage patterns. Second, chemotherapy could induce an uneven tumour response in axillary lymph node eradication. These effects would likely result in decreased SN accuracy after NAC. A few studies included both patients with an SN biopsy before/without NAC and after NAC,[38,43,48] and reported a significantly decreased SN identification rate after NAC.

When comparing SN success rates between heterogeneous studies (i.e. between studies including patients treated with NAC versus studies including patients without NAC), one must take into account that the false-negative rate depends on the probability of nodal involvement. Among patients with a lower probability of nodal involvement, there is more variation in the false-negative rate because the sample size is smaller.[58] This also holds true for the comparison of the SN false-negative rate of initially clinically node-positive patients (who have a high risk of SN and non-SN involvement) versus clinically node-negative patients. The clinical significance of an unsuccessful SN procedure on the other hand may also differ. Previous studies reported minimal impact of false-negative SNs before or without systemic therapy regarding local recurrences, but this might not hold true in this 'high risk' group treated with NAC.

In conclusion, the SN accuracy parameters following NAC are not substantially different from prior multicentre studies evaluating SN success rates without NAC. Since about half of the included patients in this review had a negative SN, this could substantially reduce the extent of axillary surgery. However, the included series contain methodological weaknesses and several features have been reported to be associated with a decreased SN accuracy after NAC in individual studies. Given the limited power for subgroup analyses, no definitive conclusions can be drawn regarding the precise value of SN biopsy in subgroups of breast cancer patients, i.e. those with clinical nodal involvement. Besides, none of the authors of the published studies on SN biopsy after NAC abandoned an ALND yet and it is unsure whether surgeons are now comfortable doing so. Awaiting further data on the safety of SN biopsy after NAC, it can be performed before NAC with the same outcome as without NAC. Nevertheless, the conclusions of this systematic review at least warrant a clinical

trial with long term follow-up to evaluate the safety of omission of an ALND in patients with a negative SN after NAC. Future advances in molecular profiling and imaging techniques may further help to determine which patients are most likely to have negative axillary nodes following a specific chemotherapeutic regimen, in which omission of an ALND could be more easily accepted for those patients with a negative SN after NAC. We believe that performing an SN biopsy following NAC could for the time being anyway be considered on an individual basis (i.e. clinically node-negative patients following NAC), but there is insufficient evidence to recommend this as a standard procedure.

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Chapter 8

Axillary staging in breast cancer patients treated with neoadjuvant chemotherapy in two Dutch phase III studies

Birgit EPJ Vriens, Kristien BMI Keymeulen, Judith R Kroep, Ayoub Charehbili, Petronella G Peer, Maaike de Boer, Maureen JB Aarts, Esther M Heuts, Vivianne CG Tjan-Heijnen on behalf of the Dutch Breast Cancer Research Group (BOOG)

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Abstract

Background

Primary aim of our study was to assess the impact of timing of sentinel node procedure, pre- versus post-neoadjuvant chemotherapy, on final pathologic node-negative rate (pN0) in patients with clinically node-negative (cN0) breast cancer. Secondary endpoint was the usability of the sentinel node procedure in patients with clinically node-positive disease that converted to cN0 after neoadjuvant chemotherapy.

Patients and methods

Patients were enrolled in two sequentially conducted Dutch phase III trials, studying the impact of two neoadjuvant chemotherapy schedules and use of zoledronic acid on complete pathologic response rate. For the present analyses, patients were excluded if they had not undergone surgical axillary staging.

Results

In total 439 patients were included, of whom 230 (52%) had pre-treatment cN0. In this group, pN0 status was seen in 58% (*N*=23) of patients with a sentinel node biopsy post-neoadjuvant chemotherapy compared to 51% (*N*=83) pre-neoadjuvant chemotherapy, including the axillary lymph node dissection whenever performed. In multivariable analysis, timing of sentinel node procedure (pre- versus post- neoadjuvant chemotherapy) was, however, not significantly associated with final pN0/pN0(i+) status, with an odds ratio of 1.18 (95% CI 0.64-2.18) after correction for age, clinical tumour status, histology, grade, hormone- and HER2 receptor. Of patients with clinically node-positive disease only 15% had a final pN0 status, with a false-negative rate of the sentinel node of 30%.

Conclusion

In breast cancer patients with cN0 disease, sentinel node procedure performed post-neoadjuvant chemotherapy led to nodal down staging, although not statistically significant after multivariate correction for patient and tumour characteristics

Introduction

Neoadjuvant chemotherapy in breast cancer patients provides the opportunity to monitor treatment effects in vivo, which may also have a positive effect on coping with the disease for the patient, and has the potential to downstage the primary tumour which may facilitate breast conserving surgery. Neoadjuvant chemotherapy has also shown to eradicate nodal disease in 20% to 40% of patients.[1-3] Obviously, if the axillary lymph nodes are truly negative, there can be no possible benefit from performing an axillary lymph node dissection. This raises the question whether the current widespread policy of performing a sentinel node procedure at initial diagnosis *pre*-neoadjuvant chemotherapy in patients with at baseline clinical node-negative disease is still appropriate. Another question is whether performing a sentinel node procedure *post*-neoadjuvant chemotherapy may potentiate axilla-conserving treatment in patients with clinically node-positive disease at initial diagnosis when converted to clinical node-negative disease.

Previously, we reported a systematic review on twenty-seven studies including 2148 patients with regard to the accuracy of sentinel node biopsy post-neoadjuvant chemotherapy.[4] The majority of the studies included both clinically node-negative and clinically node-positive patients. The pooled sentinel node identification rate, false negative rate (FNR), negative predictive value and accuracy were 90.9%, 10.5%, 89.0% and 94.4%, respectively. The reported sentinel node success rates were heterogeneous and several variables, amongst others clinical node-positivity pre-neoadjuvant chemotherapy, were reported to be associated with decreased sentinel node accuracy.

In two, quite recent, large sentinel node studies, the SENTINA study and the ACOSOG Z1071 study, it was confirmed that the sentinel node procedure post-neoadjuvant chemotherapy in patients with initially node-positive disease converted to clinically node-negative disease resulted in a lower sentinel node detection rate and higher false-negative rate compared to upfront sentinel node procedure performed in patients who had clinically node-negative disease at initial diagnosis.[5,6] Currently, in patients with clinically node-negative disease, the timing of sentinel node procedure is still a matter of debate.

Considering the above, we conclude that the most optimal timing of sentinel node procedure in patients with *clinically node-negative disease (cN0) at diagnosis* who will undergo neoadjuvant systemic therapy still remains to be elucidated. Especially patients with nodal low-volume disease may achieve a pathologic complete remission in the lymph nodes. Therefore, we re-analysed the data from two Dutch randomised phase III breast cancer studies on neoadjuvant chemotherapy with regard to final pathologic nodal status related to baseline clinical lymph node status and related to timing of axillary staging.[1,7]

Methods

Inclusion of patients

Patients were enrolled in two sequentially conducted phase III trials on neoadjuvant chemotherapy under auspices of the Dutch Breast Cancer Research Group (BOOG), the INTENS and the NEOZOTAC study.[1,7] In the INTENS study, patients were randomised between TAC and AC-T neoadjuvant chemotherapy (doxorubicin (A), cyclophosphamide (C) and docetaxel (T)).[1] In the NEOZOTAC study, patients were treated with TAC neoadjuvant chemotherapy and randomised between additional zoledronic acid or not.[7] Primary endpoint of both studies was pathologic complete response (pCR); in the INTENS study defined as pCR of the breast and in the NEOZOTAC study as pCR in breast and lymph nodes. All patients provided written informed consent before enrolment and both studies were conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Patients were eligible for these studies if clinically positive lymph nodes were present and/or the primary tumour was larger than 3 cm in size in the INTENS study and a minimum of 2 cm in the NEOZOTAC trial. Patients were excluded in the presence of distant metastasis. Of relevance, in the NEOZOTAC study patients with HER2-positive disease were excluded, whereas these could be included in the INTENS study. As a result patients included in NEOZOTAC had on average a more favourable tumour profile: smaller tumours, more node-negative, and more often ER-positive. For the present analysis, patients from both studies were considered eligible, if they had a surgical axillary staging by sentinel node procedure and/or axillary lymph node dissection.

Axillary staging in INTENS and NEOZOTAC

Baseline clinical nodal status was based on the findings obtained by physical examination and axillary ultrasound, with or without confirmation by fine needle aspiration. In patients with clinically negative lymph nodes a sentinel node procedure could be performed either before start or post-neoadjuvant chemotherapy depending on hospital policy and changing over the years.
In patients with at baseline clinical node-positive disease and a complete response in the axilla post-neoadjuvant chemotherapy (based on physical and ultrasound examination), a sentinel node procedure could be performed as an optional side study post-neoadjuvant chemotherapy, but in this situation always followed by a completion axillary lymph node dissection. In all patients who underwent a sentinel node procedure, dual agent mapping by radio colloid and Patent Blue was used.

Endpoints

The primary endpoint of the present analysis was the final pathologic node-negative rate in patients with at baseline clinically node-negative disease, that underwent

axillary staging by sentinel node biopsy with or without axillary lymph node dissection, in relation to timing pre- or post-neoadjuvant chemotherapy.
Secondary endpoint of the present study was the final pathologic node-negative rate post-neoadjuvant chemotherapy (ypN0) by sentinel node procedure and axillary lymph node dissection in patients with at baseline clinical node-positive disease that converted to clinical node-negative status. Another secondary endpoint was the false-negative rate of the sentinel node procedure in patients with at baseline node-positive lymph nodes and a clinical complete response in the axilla post-neoadjuvant chemotherapy (per protocol followed by an axillary lymph node dissection).

Definitions

The pathologic nodal status is based on the (combined) results from the sentinel node procedure and results from axillary lymph node dissection whenever performed. Final pathologic node-negative status includes both pN0(i-) and pN0(i+). Pathologic node-positive status includes pN1mi and pN1-3.[8]
The false-negative rate of the sentinel node procedure is obtained by dividing the number of patients who were sentinel node negative but non-sentinel node positive by the number of patients who had a positive sentinel node or non-sentinel node, that is (1 – sensitivity).[9] Non-sentinel nodes were defined as lymph nodes obtained during the completion axillary lymph node dissection.

Statistics

For clinically node-negative patients at baseline, the effect of the timing of the sentinel node procedure on the combined outcome of sentinel node procedure and axillary lymph node dissection was addressed in a logistic regression model correcting for potentially confounding factors, that is, age at diagnosis, cT-status, histology, tumour grade, hormone receptor status and HER2-status. This yielded the adjusted odds ratio (OR) with 95% confidence interval.

Results

Patient inclusion

Between February 2006 and May 2012, a total of 448 patients were enrolled in the Dutch INTENS (*N*=202) and NEOZOTAC (*N*=246) phase III trials. Of these, 9 patients did not undergo a sentinel lymph node procedure or axillary lymph node dissection and were, therefore, excluded. Of the 439 included patients, 230 (52%) had pre-treatment clinically node-negative disease and 209 (48%) node-positive disease, based on physical and ultrasound examination with or without cytology (Figure 8.1).

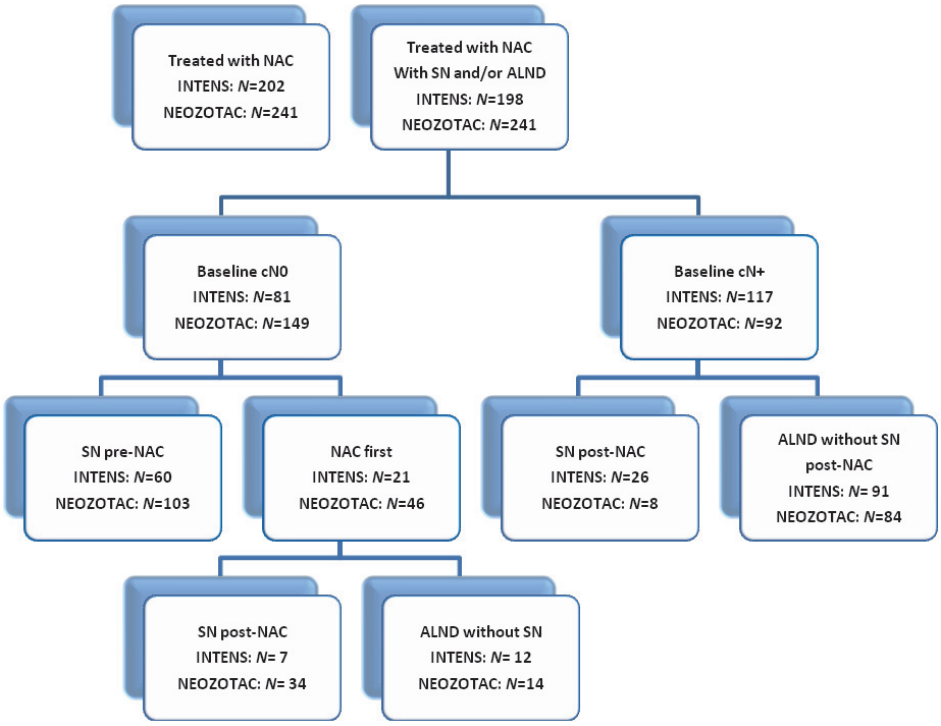

Figure 8.1 Consort diagram of axillary staging during neo-adjuvant chemotherapy in two Dutch phase III studies, the INTENS and NEOZOTAC study. Abbreviations: SN, sentinel node; ALND, axillary lymph node dissection; NAC, neo-adjuvant chemotherapy; Baseline cN0 based on ultrasound plus or minus fine needle aspiration; Patients who underwent both SN pre-NAC and post-NAC were classified under SN pre-NAC.

Of patients with at baseline clinically node-negative disease, a sentinel node procedure pre-neoadjuvant chemotherapy was done in 163 patients (71%) and axillary staging post-neoadjuvant chemotherapy in 67 patients (29%). Patients with a sentinel node pre-neoadjuvant treatment had a smaller tumour and more often of ductal histology compared to those with axillary staging with or without sentinel node post-neoadjuvant chemotherapy, but a larger tumour of lower grade when compared to those who underwent a sentinel node procedure post-neoadjuvant chemotherapy (Table 8.1).

As could be expected, patients with clinically node-positive disease had more unfavourable primary tumour characteristics compared to patients with clinical node-negative breast cancer (Table 8.1). In patients with clinically node-positive disease, the tumours were larger, more often of ductal histology, grade III, ER negative and/or HER2 positive.

Table 8.1 Characteristics of patients who underwent a sentinel node procedure pre-neoadjuvant chemotherapy or who first received neoadjuvant chemotherapy before axillary staging, categorized by baseline clinical nodal stage.

	Baseline cN0				Baseline cN+		
	SN pre-NAC	NAC first			NAC first		
		Total	SN post-NAC	ALND without SN	Total	SN post-NAC	ALND without SN
	N=163 (%)	*N*=67 (%)	*N*=41 (%)	*N*=26 (%)	*N*=209 (%)	*N*=34 (%)	*N*=175 (%)
Age (years)							
≤ 50	97 (60)	41 (61)	23 (56)	18 (69)	127 (61)	20 (59)	107 (61)
> 50	66 (40)	26 (39)	18 (44)	8 (31)	82 (39)	14 (41)	68 (39)
Median (range)	48 (29 -68)	49 (33 – 65)	50 (33 - 65)	49 (34 – 62)	49 (24 – 70)	49 (36 – 70)	49 (24 – 70)
cT-status							
cT1-2	106 (65)	36 (54)	30 (73)	6 (23)	99 (47)	25 (74)	74 (42)
cT3-4	57 (35)	31 (46)	11 (27)	20 (77)	110 (53)	9 (26)	101 (58)
Histology							
Ductal	121 (77)	43 (68)	30 (73)	13 (57)	171 (86)	28 (88)	143 (86)
Lobular	26 (16)	16 (25)	8 (20)	8 (35)	16 (8)	3 (9)	13 (8)
Other	11 (7)	5 (8)	3 (7)	2 (9)	11 (6)	1 (3)	10 (6)
Unknown	5	3	0	3	11	2	9
Histological grade							
Grade I	30 (26)	10 (25)	5 (24)	5 (26)	19 (12)	3 (10)	16 (12)
Grade II	66 (58)	21 (53)	10 (48)	11 (58)	76 (48)	14 (48)	62 (48)
Grade III	18 (16)	9 (22)	6 (29)	3 (16)	63 (40)	12 (41)	51 (40)
Unknown	49	27	20	7	51	5	46
Hormone receptor status							
Positive	131 (80)	53 (79)	33 (80)	20 (77)	147 (70)	26 (76)	121 (69)
Negative	32 (20)	14 (21)	8 (20)	6 (23)	62 (30)	8 (24)	54 (31)
HER2 status							
Positive	11 (7)	2 (3)	1 (2)	1 (4)	31 (15)	10 (29)	21 (12)
Negative	152 (93)	65 (97)	40 (98)	25 (96)	178 (85)	24 (71)	154 (88)
Hormone / HER2 status							
Both negative	29 (18)	13 (19)	7 (17)	6 (23)	47 (22)	4 (12)	43 (25)
Study							
INTENS	60 (37)	21 (31)	7 (17)	14 (54)	117 (56)	26 (76)	91 (52)
NEOZOTAC	103 (63)	46 (69)	34 (83)	12 (46)	92 (44)	8 (24)	84 (48)
Axillary treatment							
ALND	73 (45)	39 (58)	13 (32)	26 (100)	207 (99)	32 (94)	175 (100)

SN, sentinel node; NAC, neoadjuvant chemotherapy; ALND, axillary lymph node dissection; Baseline cN0 and cN+ based on ultrasound plus or minus fine needle aspiration; Patients who underwent both SN pre-NAC and post-NAC were classified under SN pre-NA(C).

Baseline node-negative

Of all patients with at baseline clinically node-negative disease, 52% had a (final) pathologic node-negative status, including the axillary lymph node dissection whenever performed (Table 8.2). Compared to patients with a sentinel node procedure pre-neoadjuvant chemotherapy, those with a sentinel node procedure post-neoadjuvant chemotherapy had more often a pathologic node-negative status, 58% (*N*=23) versus 51% (*N*=83) including the axillary lymph node dissection and 59% (*N*=23) versus 53% (*N*=86) based on sentinel node procedure alone (Table 8.2). A (final) pathologic node-negative status was seen in 56% (5/9) of patients with hormone receptor positive / HER2 positive breast cancer, in 48% (84/175) of cases with a hormone receptor positive / HER2 negative tumour, in 0% (0/4) of cases with a hormone receptor negative / HER2 positive tumour and in 74% (31/42) of cases with a hormone receptor negative / HER2 negative (triple negative) breast tumour.
Timing of sentinel node procedure (post- versus pre-neoadjuvant chemotherapy) was not significantly associated with final pN0/pN0(i+) status with an OR of 1.18 (95% CI 0.64-2.18) after correction for age, clinical tumour status, histology, histological grade, hormone receptor and HER2 status (Table 8.3).
Of the 77 patients with sentinel node negative disease who did not undergo completion axillary lymph node dissection, 28 were included in the INTENS study. With a median follow up of 6 years three patients had distance recurrence and none had a regional recurrence. In the NEOZOTAC trial, follow-up duration is still too short to assess 5-year recurrence or survival rates.

Baseline node-positive

Of patients with initially node-positive disease and a sentinel node procedure post-neoadjuvant chemotherapy because of a complete remission based on imaging evaluation (*N*=34), 39% had a negative sentinel node. In all but one a complete axillary lymph node dissection was done. When including the results of axillary lymph node dissection, only 15% could still be classified as pathologic node-negative (Table 8.2).
For patients with initially node-positive disease who had a sentinel node procedure and completion axillary lymph node dissection post-neoadjuvant chemotherapy (*N*=32), the false-negative rate of the sentinel node procedure was 30%.
Of all patients with initially node-positive disease, 23% had a pathologic node-negative status (Table 8.2).

Table 8.2 Primary and secondary endpoints of patients who underwent a sentinel node procedure pre-neoadjuvant chemotherapy or who first received neoadjuvant chemotherapy before axillary staging, categorized by baseline clinical nodal stage.

		Baseline cN0			Baseline cN+		
	SN pre-NAC	NAC first			NAC first		
		Total	SN post-NAC	ALND without SN	Total	SN post-NAC	ALND without SN
	N=163 (%)	*N*=67(%)	*N*=41 (%)	*N*=26 (%)	*N*=209(%)	*N*=34 (%)	*N*=175 (%)
Pathological nodal status (SN)							
pN0(i-)/pN0(i+)	86 (53)		23 (59)			13 (39)	
pN1mi	20 (12)		3 (8)			7 (21)	
pNmacro	57 (35)		13 (33)			13 (39)	
uk	0		2			1	
Pathological nodal status (SN + ALND)							
pN0(i-)/pN0(i+)	83 (51)	37 (56)	23 (58)	14 (54)	48 (23)	5 (15)	43 (25)
pN1mi	19 (12)	5 (8)	3 (7)	2 (8)	30 (14)	6 (18)	24 (14)
pN1macro	61 (37)	24 (36)	14 (35)	10 (38)	129 (62)	22 (65)	107 (61)
uk	0	1	1	0	2	1	1

SN, sentinel node; ALND, axillary lymph node dissection; NAC, neoadjuvant chemotherapy; Baseline cN0 and cN+ based on ultrasound plus or minus fine needle aspiration; Patients who underwent both SN pre-NAC and post-NAC were classified under SN pre-NAC; uk, unknown.

Table 8.3 Impact of timing of axillary stage pre- versus post-neoadjuvant chemotherapy in patients with clinical node-negative disease that underwent a sentinel node procedure on final pN0/pN0(i+) status. Unadjusted odds ratio (OR), OR adjusted for one of the covariates age, cT status, histology, tumour grade, hormone receptor status or HER 2 status and the adjusted OR for all previously mentioned covariates.

		OR	95% CI	*N*
Unadjusted OR		1.23	0.69 – 2.19	229
Adjusted OR for 1 covariable	Age (≤ 50y/>50y)	1.25	0.70 – 2.22	229
	cT –status (cT1-2/ cT3-4)	1.26	0.70 – 2.24	229
	Histology (ductal/lobular/other)	1.17	0.64 – 2.13	221
	Grade (G1/G2/G3)	1.28	0.61 – 2.69	154
	Hormone receptor (positive/negative)	1.22	0.68 – 2.18	229
	HER2-status (positive/negative)	1.20	0.68 – 2.15	229
Adjusted OR for all covariables		1.18	0.64 – 2.18	154

Discussion

We performed a combined analysis on two sequentially conducted phase III trials using neoadjuvant chemotherapy in The Netherlands in the years 2006-2012. The main results of the two studies on impact of type of chemotherapy and role of zoledronic acid as an adjunct to neoadjuvant chemotherapy on pathologic complete remission rate in the breast and/or breast and lymph nodes have been reported before.[1,7] In the two studies, the timing of axillary staging was largely depended on the baseline clinical nodal status, although in patients with clinical node-negative disease the sentinel node procedure was increasingly used post-neoadjuvant chemotherapy in later years. Our findings show that axillary lymph node dissection post-neoadjuvant chemotherapy, compared to axillary staging pre-neoadjuvant chemotherapy, resulted more frequently in a final pathologic node-negative status (58% versus 51%) in patients with at baseline clinical node-negative disease, although this difference remained not statistically significant different after correction for age, clinical tumour status, histology, histological grade, hormone receptor and/or HER2 status. The absence of regional recurrences after a median follow-up of six years in those who did not undergo completion axillary lymph node dissection underscores the conclusion that performing the sentinel node procedure post-chemotherapy is safe in patients with clinically node-negative breast cancer at initial diagnosis.

The optimal timing of the sentinel node procedure with respect to neoadjuvant chemotherapy has almost exclusively been studied in patients who had initially clinical node-positive disease. Various meta-analyses, the recent prospective SENTINA cohort study and the ACOSOG Z1071 study have all shown a high false-negative rate in this particular subgroup of patients.[5,6] In the SENTINA trial a false negative rate of 14.2% was reported and 24% if only 1 sentinel node was removed. ACOSOG Z1071 reported

a false negative rate of 12.6% (in 81% of the patients two or more sentinel lymph nodes were removed). In our study, we also observed a high false-negative rate of 30% in patients with initially node-positive disease that converted to clinical node-negative disease. In 48% of these patients two or more sentinel nodes were removed. In the studies reported so far, long-term outcome of patients who converted from clinical-positive to sentinel node-negative without completion axillary lymph node dissection is not available. Especially for patients with triple negative disease who currently have no additional systemic therapy options outside a clinical trial setting after use of neoadjuvant chemotherapy, one can imagine that there might be an increased risk for regional recurrence if positive non-sentinel nodes are left behind. In our study, four patients with triple negative breast cancer converted from clinical positive to negative and underwent both a sentinel node procedure and completion axillary lymph node dissection. Of these, two had a positive sentinel node, and two had a negative sentinel node with no non-sentinel node metastases (i.e., no false-negative sentinel nodes).

Nevertheless, to be able to further de-escalate axillary treatment we should think of new ways to identify the nodal status of patients and to use new techniques or combination of existing techniques. It is well-known, that the reliability of the sentinel node procedure is enhanced by using both radiolabeled colloid and methylene blue (false-negative rate 10.8%) and by removing a higher number of nodes (false negative rate 9.1%).[5,6,10] An alternative method is the use of a marker, e.g. radioactive iodine seed, selectively placed into the clinically most suspected positive-node.[11] In the study of Donker *et al.* the identification rate of the marked node with a radioactive iodine seed was 97% with a false negative rate of 7%.[11] In 5 of 30 patients with a negative marked node additional positive lymph nodes were found (negative predictive value of 83% (95% CI 65% – 94%)). This negative predictive value is slightly lower compared to the data in our systematic review of the accuracy of sentinel node biopsy post-neoadjuvant chemotherapy with a pooled negative predictive value rate of 89% (95% CI 85 – 92).[4] Nathanson *et al.* reported a correlation between the marked nodes (clinical node negative or node positive disease) identified on initial ultrasound of the axilla and sentinel lymph node(s) of 78%.[12] Boughey *et al*. reported that a clip was placed in 170 patients with node positive disease who underwent a sentinel node procedure and axillary lymph node dissection after neoadjuvant chemotherapy.[13] The clip was found as a sentinel node in 107 patients, in the lymph node dissection specimen in 34 patients, and the location of the clipped node was unknown in 29 patients. A combination of sentinel node procedure and a marked node procedure (e.g. radioactive iodine seed, clip placement or preoperative tattooing of the clinical positive-node) in patients with initially clinical node-positive disease might be an interesting new strategy to reduce extend of axillary surgery after neoadjuvant chemotherapy.[11,13,14] Boughey *et al.* reported a false negative rate of 6.8% (95% CI 1.9-16.5%) in patients with clip placement of the initially clinical node positive disease

and a sentinel node procedure after neoadjuvant chemotherapy with removal of at least 2 sentinel nodes followed by axillary dissection.[13] Caudle *et al*. reported in a prospective single-institution study with 208 breast cancer patients a false negative rate of 10.1% (95% CI 4.2-19.8) when a sentinel node procedure was done after neoadjuvant chemotherapy, evaluation of the clipped node resulted in a false negative rate of 4.2% (95% CI 1.4-9.5) and the combination of sentinel node and a clipped node reduced the false negative rate to 1.4% (95% CI 0.03-7.3).[15] Whether the combination procedures are technically feasible in daily practice and whether the negative predictive failure improves needs to be studied more intensively before implementing this technique in daily practice.

Our findings show that the number of patients requiring axillary treatment is reduced by performing the sentinel procedure post-neoadjuvant chemotherapy in patients with clinically node-negative disease. In a single centre study, a total of 3746 patients with clinical T1-T3 node-negative breast cancer underwent a sentinel node procedure from 1994 to 2007, of whom 575 post-neoadjuvant chemotherapy.[16] Sentinel node procedure post-chemotherapy resulted in fewer patients with positive sentinel lymph nodes (absolute reduction of 6.3% for T1, 16% for T2 and 21% for T3 tumours) and decreased the number of axillary dissections in patients with T2 (27% vs. 41%, p=0.0001) and T3 tumours (45% vs. 66%, p=0.045). There was no difference in regional recurrence rates, after adjusting for clinical stage (0.9% in patients with a sentinel node procedure pre-chemotherapy versus 1.2% post-chemotherapy). Recently, a population based study also reported an increased proportion of patients with a negative sentinel node when assessed post- compared to pre-chemotherapy (67% versus 54%; *p*=0.001).[17] The post-neoadjuvant chemotherapy sentinel node procedure was also associated with significantly less frequent axillary treatment in this study. Reduced axillary treatment may result in less arm and shoulder morbidity as has been reported in the AMAROS trial.[18]

A limitation of our and aforementioned studies is that it concerned observational studies. Actually, our study concerned a non-randomised analysis within a randomised frame primarily testing the impact of type of chemotherapy (AC-T versus TAC) in the INTENS study and of zoledronic acid in the NEO-ZOTAC study on pathologic complete remission rate. As in- and exclusion criteria of both studies were largely the same, we were able to include a rather uniformly selected patient group for the current research question. Because of the descriptive post-hoc design of the present study we were, however, not able to address the underlying reasons for timing and type of axillary staging in patients with clinically node-negative disease, although we believe we have taken possible confounding factors like tumour size into account by the multivariable analysis. Actually, a trial randomizing between sentinel node procedure pre- versus post- neoadjuvant chemotherapy would provide the highest level of evidence. As far as we know, such a trial is not being conducted and will probably not

be done in the future, as one may be convinced by the existing evidence from aforementioned observational studies.

It is likely that response to neoadjuvant chemotherapy predicts for the potential benefit of otherwise adjuvant delivered treatment in terms of improved survival. Based on our and other studies, we conclude that in breast cancer patients with clinically node-negative disease the sentinel node procedure is preferentially postponed till after the end of neoadjuvant chemotherapy to take maximum benefit of its effect on nodal down staging. For patients with initially node-positive disease that convert to clinical node-negative disease after neoadjuvant therapy, techniques for more reliable identification of possibly involved nodes needs yet further testing.

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Chapter 9

Summary, general discussion and future perspectives

Summary

Breast cancer is currently the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide.[1] Over the past decade we have seen a significant decrease of mortality of early breast cancer due to early detection, multidisciplinary approach and improved treatment options, i.e., chemotherapy, HER2 targeted therapy and endocrine therapy.[2] With the addition of taxanes to anthracyclines and cyclophosphamide in recent years, overall survival has clearly further improved.
Chemotherapy can be applied in the neoadjuvant or adjuvant setting. Neoadjuvant chemotherapy is an effective alternative to adjuvant chemotherapy in both early and locally advanced breast cancer.[3] Neoadjuvant therapy can help to optimize chemotherapy schemes and drug combinations to achieve higher pathologic complete response (pCR) rates and to better understand tumour biology by in vivo responsiveness testing. It may also allow assessment of efficacy of treatment in a shorter time period of months rather than years of follow-up.

This thesis addresses several aspects of neoadjuvant chemotherapy in breast cancer patients in relation to efficacy, response assessment and axillary strategy. In **chapter 1** a general introduction and outline of the thesis is presented. The first part of this thesis focuses on efficacy of taxane based chemotherapy schedules and the possible protective effect of prior G-CSF use or of a prior chemotherapy cycle on myelotoxicity in the subsequent chemotherapy cycle. The second part of this thesis addresses different methods of response assessment, accuracy of the sentinel node biopsy after neoadjuvant chemotherapy and the impact of timing of axillary staging.
The results are mainly based on the data of the Dutch phase III INTENS study, comparing sequential use of docetaxel after anthracyclines and cyclophosphamide (AC-T) and the upfront use of the triple combination of docetaxel with anthracyclines and cyclophosphamide (TAC). Moreover, the data presented in this thesis are based on the national, phase III Two-to-Six study[4] and the NEO-ZOTAC study.[5] In the Two-to-six study breast cancer patients were randomized to primary G-CSF prophylaxis during all six TAC-containing chemotherapy cycles or primary prophylaxis limited to the first two chemotherapy cycles only. The NEO-ZOTAC trial studied the impact of neoadjuvant chemotherapy (TAC) and use of zoledronic acid on pCR rate.

Research questions in the thesis

1. Are the chosen comparator and/or study design in taxane-containing chemotherapy schemes in metastatic and early breast cancer trials of influence on efficacy endpoints? **Chapter 2**
2. Is there a difference in the pCR rate in the breast in patients with newly diagnosed non-metastatic breast cancer comparing two different taxane-containing neoadjuvant chemotherapy schemes? **Chapter 3**

3. Is there a difference in 5-year disease-free and overall survival in patients with newly diagnosed non-metastatic breast cancer comparing two different taxane-containing neoadjuvant chemotherapy schemes? **Chapter 4**
4. Is there is a protective effect of a prior docetaxel-containing chemotherapy cycle or prior granulocyte-colony stimulating factor (G-CSF) prophylaxis on the next cycle blood cell counts? **Chapter 5**
5. Is there a difference in accuracy of clinical breast tumour size measurement post-neoadjuvant chemotherapy by magnetic resonance imaging or ultrasound? **Chapter 6**
6. What is the accuracy of sentinel node biopsy post-neoadjuvant chemotherapy? **Chapter 7**
7. What is the impact of timing of axillary staging before versus after neoadjuvant chemotherapy on final pathologic node-negative rate in patients with clinically node-negative breast cancer? **Chapter 8**

In recent years, new drugs have shown activity in metastatic breast cancer, but not always resulting in an overall survival benefit. The anthracyclines and taxanes, docetaxel and paclitaxel, represent the most potent drugs for use in breast cancer. Taxanes did not improve survival in metastatic breast cancer trials, whereas they did so in early breast cancer trials.[6,7] In chapter 2 we present a systematic review of phase III taxane-based chemotherapy studies in early and metastatic breast cancer to assess which factors may have contributed to the observed differential outcome. In total, 10 trials in the metastatic breast cancer setting and 21 trials in the adjuvant breast cancer setting were included. In the metastasized setting no improvement in progression-free and overall survival was seen for the taxane containing treatment. In contrast to the pooled analysis of early breast cancer trials, which showed a significant difference in favour of adding a taxane for both disease-free survival (hazard ratio 0·85; 95% CI 0·80-0·91) and overall survival (hazard ratio 0·85; 95% CI 0·79-0·91). In the majority of the metastatic breast cancer studies taxanes were substituting other active cytotoxic drugs, mainly cyclophosphamide, whereas in early breast cancer, many studies focused on the impact of taxanes when delivered at full dose in addition to anthracyclines, instead of substitution. We conclude that the negative results of taxanes in metastatic breast cancer studies seem to be caused by the design of the randomized trial.

It is accepted worldwide that taxanes should somehow be incorporated in the (neo-) adjuvant treatment of breast cancer patients at increased risk of relapse. The most optimal strategy for incorporating docetaxel is, however, still under investigation. In **chapter 3** we report the main results of the INTENS study, which was designed to determine whether delivering neo-adjuvant taxane-based chemotherapy at a higher dose in a shorter period of time would improve the outcome of early breast cancer patients. In total, 201 patients were included. 21% of patients treated with sequential

AC-T chemotherapy achieved a pCR in the breast as compared to 16% of patients treated with concurrent TAC-chemotherapy (odds ratio 1.44; 95% CI 0.67-3.10), when considering central pathology review results. AC-T without primary G-CSF prophylaxis was associated with more febrile neutropenia episodes as compared to TAC with primary G-CSF prophylaxis (23% versus 9%), and with more grade 3/4 sensory neuropathy (5% versus 0%). We concluded, that no statistically significant differences in pCR rates were observed between the two treatment arms consisting of the same drugs but at a different schedule and different cumulative dose. And, that to a great extent the differential toxicity profile could be explained by different use of primary G-CSF prophylaxis.

After a median follow up of 6 years we reported the disease-free survival and overall survival in chapter 4. For patients in the AC-T arm the 5-year disease free survival was 81% and for patients who received TAC chemotherapy 71% (log-rank P=0.015). Five-year overall survival was also significantly superior for the AC-T arm: 84% versus 76%, respectively (log-rank P=0.041). Hence, we concluded that sequentially delivered AC-T chemotherapy seemed to outperform concomitantly delivered TAC chemotherapy in terms of improved 5-year disease free and overall survival.

An anthracycline-taxane chemotherapy regime is effective in breast cancer, but it is also very myelotoxic with a substantial risk of febrile neutropenia (FN). In **chapter 5** we analysed if a prior chemotherapy cycle or use of primary G-CSF prophylaxis during a prior chemotherapy cycle had a myeloprotective effect in subsequent chemotherapy cycles. For this study we used data from the Dutch Two-to-Six study, a randomized study in patients with early stage breast cancer and increased risk of febrile neutropenia during neoadjuvant or adjuvant chemotherapy. We investigated the nadir blood cell counts of all cycles in the patients treated with primary G-CSF prophylaxis throughout all chemotherapy cycles (G-CSF 1-6 arm) and the nadir blood cell counts of cycles 3-6 in the patients treated with primary G-CSF prophylaxis during the first two cycles only (G-CSF 1-2 arm). In the G-CSF 1-6 arm the median nadir of the absolute neutrophil count decreased from 7.1 x 10^9/l in cycle 1 to 5.5 x 10^9/l in cycle 6. During treatment in the G-CSF 1-2 arm, when primary G-SCF prophylaxis was discontinued, the nadir absolute neutrophil count showed a persistent grade 4 neutropenia of 0.1 x 10^9/l in cycles 3 to 6. Hence, we conclude that there is no protective effect of prior G-CSF or of the prior chemotherapy cycle on myelotoxicity in the subsequent chemotherapy cycles.

In **chapter 6** we evaluated the accuracy of clinical imaging by magnetic resonance imaging and ultrasound of the primary breast tumour after neoadjuvant chemotherapy related to the post-operative histological tumour size (gold standard), and whether this varied with breast cancer subtype. Patients enrolled in the INTENS study of whom clinical imaging was available after the neoadjuvant chemotherapy

and known histopathological tumour size were included in this analysis. Magnetic resonance imaging estimated residual tumour size with ≤10 mm discordance in 54% of patients, overestimated tumour size with >10 mm in 28% and underestimated tumour size with >10 mm in 18% of patients. With ultrasound these figures were 63%, 20% and 17%, respectively. The negative predictive value in hormone receptor-positive tumours for both magnetic resonance imaging and ultrasound was low, 26% and 33%, respectively. In this study**,** ultrasound was at least as good as breast magnetic resonance imaging in providing information on residual tumour size after neoadjuvant chemotherapy. However, both modalities suffered from a substantial percentage of over-and underestimation of tumour size and in addition both showed a low negative predictive value of pCR.

Chapter 7 provides an overview of the current literature to determine the accuracy of a sentinel node biopsy after neoadjuvant chemotherapy. Twenty-seven studies were included in this review with a total study population of 2148 patients. We found a pooled sentinel node identification rate of 90.9% (95% CI 88.0-93.1) and a false-negative rate of 10.5% (95% CI 8.1-13.6). A majority of studies included both clinically node-negative and node-positive patient before start of neoadjuvant chemotherapy. Factors such as primary tumour size and clinical nodal involvement before and/or after neoadjuvant chemotherapy have been reported to affect the accuracy of the sentinel node biopsy. In 2009, we concluded that there might be a potential role for performing a sentinel node procedure following neoadjuvant chemotherapy, which could be considered on an individual basis, but that there was yet insufficient evidence to recommend this as a standard procedure. We recommended further research with subgroup analysis using variables reported to be associated with decreased sentinel node accuracy in order to clearly define its value in the subgroups of breast cancer patients.

In **chapter 8** we assessed the impact of timing of axillary staging before versus after neoadjuvant chemotherapy on final pathologic node-negative rate in two sequentially conducted Dutch phase III trials, the INTENS and NEOZOTAC trial. In the INTENS and NEOZOTAC studies, the impact of different neoadjuvant chemotherapy schedules and of zoledronic acid as an adjunct to neoadjuvant chemotherapy on pCR rate was primarily assessed. The research question regarding the most optimal timing of the sentinel node procedure was a secondary study endpoint. The results of this secondary endpoint are presented in this chapter, by a combined analysis of both studies. For this substudy, patients were included if they had a surgical axillary staging by sentinel node procedure and/or axillary lymph node dissection. Of the included patients, 230 (52%) had pre-treatment clinically node-negative disease. In this group a pathologically node-negative status was seen in 58% of patients with a sentinel node procedure post-neoadjuvant chemotherapy compared to 51% of patients with a sentinel node procedure pre-neoadjuvant chemotherapy including the results of the

axillary lymph node dissection. In multivariable analysis, timing of sentinel node procedure (pre- versus post- neoadjuvant chemotherapy) was, however, not significantly associated with final pN0/pN0(i+) status, with an odds ratio of 1.18 (95% CI 0.64-2.18) after correcting for age, clinical tumour status, histology, grade, hormone- and HER2 receptor. Of patients with clinically node-positive disease only 15% had a final pathologic node-negative status, with a false-negative rate of the sentinel node of 30%. We concluded that in early-stage breast cancer patients with clinically node-negative disease, a sentinel node procedure performed after neoadjuvant chemotherapy led to nodal down staging, although not statistically significant after multivariate correction for patient and tumour characteristics. In patients with clinical node-positive disease, nodal down staging to node-negative disease was seldomly achieved with a clinical relevant high false-negative rate.

Of note, in chapter 8 we further observed a very low axillary recurrence rate in patients with sentinel node negative disease who did not undergo completion axillary lymph node dissection, after a 5 year follow up period. Of the 77 patients with sentinel node negative disease who did not undergo completion axillary lymph node dissection, 28 were included in the INTENS study. With a median follow up of 6 years three patients had distance recurrence and none had a regional recurrence. In the NEOZOTAC trial, follow-up duration is still too short to assess 5-year recurrence or survival rates.

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General discussion and future perspectives

Most of the chapters in this thesis are derived from data from the phase III neoadjuvant INTENS-study. This study was designed to determine whether delivering neo-adjuvant taxane-based chemotherapy at a higher dose in a shorter period of time indeed provided the best outcome of early breast cancer patients. Of note, at the time the study started concurrent TAC (docetaxel, doxorubicin and cyclophosphamide) chemotherapy consisting of six 3-weekly cycles was considered to be the standard treatment regime in the Netherlands and in many other European countries, whereas sequential AC-T chemotherapy consisting of eight 3-weekly cycles was considered the standard regime in many hospitals in the United States of America.
In the INTENS study, both regimens showed to be equally effective in achieving a pathologic complete response (pCR). After a median follow-up of six years, however, sequentially delivered AC-T showed a superior disease-free and overall survival for all patient subgroups compared to concurrent TAC chemotherapy. Our results support the outcome of other (neo)adjuvant trials that sequential treatment improved outcome for patients with early breast cancer.

Both the adjuvant breast cancer BIG 2-98 and the NSABP-B30 trials, comparing sequential versus concurrent use of taxanes, resulted in a better disease-free survival for the sequential arm.[1,2] The BIG 2-98 trial compared six cycles of sequentially delivered A-T with four cycles of concurrently delivered AT chemotherapy, both after treatment with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) chemotherapy, resulting in a HR of 0.83 (95% CI 0.69 – 1.00) for disease-free survival in favour of the sequential treatment arm.[1] The NSABP-B30 trial compared AC-T for eight cycles to TAC for four cycles, again showing an improved disease-free survival for the sequential arm with a hazard ratio of 0.83 (95% CI 0.73-0.95).[2] In contrast, in the large adjuvant BCIRG-005 trial eight cycles of AC-T did not improve disease-free survival when compared to six cycles of TAC (HR 1.00; 95% CI 0.86-1.16).[3,4] Apparently, the number of chemotherapy cycles, dose per cycle and frequency of chemotherapy delivery all matter. In our study, each drug was given at a higher dose per cycle (dose-intensity), but at a lower cumulative dose in the sequential treatment arm as compared to the concurrent triplet treatment arm. Patients in the concurrent TAC arm received a cumulative dose of 450 mg/m^2 docetaxel compared to 400 mg/m^2 for patients in the sequential AC-T arm and a cumulative dose of 300 mg/m^2 doxorubicin in the TAC arm compared to 240 mg/m^2 for patients in the sequential AC-T arm. Taking all the results into account, we can at least conclude that sequentially delivered chemotherapy was always superior or comparable, but never inferior, to concurrently delivered triplet chemotherapy. Moreover, the lower cumulative anthracycline dose with AC-T is attractive because of the lower risk of cardiotoxicity and risk of leukemia, and lower associated costs for imaging, treatment and follow up.

Cardiotoxicity is reported in patients who received more than approximately 250-300 mg/m^2 of anthracycline.[5,6] Cardiotoxicity as late side effect for patients treated with anthracyclines is a matter of growing concern because of the increasing number of survivors. In our study we found that both treatments had a manageable toxicity profile during therapy. Febrile neutropenia was the most frequent side effect, seen in 23% of patients treated with AC-T and in 9% treated with TAC. Of note, if febrile neutropenia occurred during treatment with AC-T, this was mainly seen during the four cycles of docetaxel monotherapy without primary G-CSF prophylaxis, which actually falls in the EORTC category of moderate increased risk (10-20%).[7] Also, the reduced use of G-CSF during AC-T therapy, for only four cycles, in comparison with the use of G-CSF during all 6 cycles of TAC chemotherapy, can be considered as an advantage for sequentially delivered chemotherapy. To prevent febrile neutropenia in the AC-T arm we now routinely recommend to offer primary G-CSF prophylaxis during the 4 cycles of docetaxel monotherapy in selected patients (≥65 years of age). In addition, we found no protective effect of G-CSF on myelotoxicity in subsequent chemotherapy cycles. Hence, it is concluded that decreased G-CSF use, together with the lower cumulative dose per agent makes sequential AC-T the most cost-effective approach with least risk of long term toxicities.

Considering the fact that in our study sequential chemotherapy had a superior outcome and in other trials sequential treatment was never found inferior to concurrently delivered triplet chemotherapy, and with lower costs and lower risk of long-term toxicity, we decided to implement the sequential schedule in our daily practice. And, based on the recent results from the international E1199, where after four AC cycles twelve weekly paclitaxel infusions showed to be superior over four 3-weekly docetaxel infusions in patients with triple negative breast cancer,[8] we amended our daily practice for this particular subgroup of patients. For patients with HER2 positive disease sequential therapy combined with anti-HER2 therapy was already implemented before.[9]

In the INTENS study 66% of patients had ER and/or PR positive disease, 20% HER2-positive disease and 25% triple negative disease. All these patient subgroups benefitted from the sequentially delivered eight cycles of treatment as compared to those treated with the triplet schedule. In recent years, we learn more about tumour response and the effect of neoadjuvant chemotherapy in breast cancer patients. Neoadjuvant chemo(immune)therapy leads to a breast tumour and nodal downstaging, especially in human epidermal growth factor receptor (HER2)-positive and triple negative cancers (i.e., ≥40% pCR), but is limited in luminal (estrogen (ER) positive / HER2-negative) cancers (i.e., 10-15% pCR).[10] pCR is often used as a surrogate endpoint for prediction of long-term clinical benefit, such as disease-free survival, and overall survival. In our study, addressing all biomarker subtypes, there was no significant difference in pCR rate for AC-T versus TAC. After a median follow-up of six

years, we yet showed that patients receiving AC-T had a superior survival for all patient subgroups as compared to TAC. Of relevance, more than half of the patients in our study had ER positive / HER2 negative disease. Moreover, we noticed that these patients actually had an excellent 5-year disease-free survival, even without pCR. This is in line with other studies showing that despite the lower pCR rates in this population, these nonetheless have a more favourable long-term prognosis. pCR is thus a poor predictor of clinical benefit in this population and drug-efficacy may be overall underestimated.
A recent meta-analysis showed a differential association between pCR and event-free survival among cancer subtypes with highest prognostic value in aggressive tumours.[11] In the HER2+ subtype, there is evidence suggesting that pCR might, however, be a surrogate endpoint for survival, with best outcome occurring in hormone receptor (HR)-negative tumours.[11,12] Nevertheless pCR is a favourable prognostic factor for the individual patient and the intensity for subsequent therapy and related toxicity.

In 2013 the Food and Drug Administration (FDA) granted accelerated approval to pertuzumab for its use in combination with trastuzumab and docetaxel as neoadjuvant treatment of patients with HER2 positive early staged breast cancer, because it nearly doubled the pCR rate.[13] They concluded that a large improvement in pCR rate based upon analysis of a full intent-to-treat population was reasonably likely to predict clinical benefit. They emphasised that a confirmatory trial should be ongoing at the time of accelerated approval. Recently, the data of the *adjuvant* Aphinity trial in which pertuzumab was added to chemotherapy and trastuzumab, were reported.[14] The results with regard to the primary endpoint were statistically significant, but the size of the treatment effect of adjuvant pertuzumab was rather disappointing. With a median follow up period of 45.4 months the 3-year rate of invasive disease-free survival was 94.1 % in the pertuzumab group and 93.2% in the placebo group resulting in a HR of 0.81 (95% CI 0.66-1.00, P=0.045) in favour of pertuzumab, with an absolute 0.9% lower rate of recurrence or death at 3 years. This treatment effect was most detectable among patients with lymph-node involvement (an absolute 1.8% lower rate of recurrence or death at 3 years) or hormone-receptor negativity (an absolute 1.6% lower rate of recurrence or death at 3 years) but the effect was statistically homogenous throughout all subgroups.
Discordant results with dual HER2 blockade in the neoadjuvant and adjuvant setting were also seen in the NeoALTTO and ALTTO trial.[15,16] In the neoadjuvant NeoALTTO trial adding lapatinib to trastuzumab resulted in a near doubling of the pCR rate,[15] but the *adjuvant* ALTTO trial showed only a small non-significant benefit for adding lapatinib to trastuzumab in the adjuvant treatment for HER2-positive breast cancer leading to withdrawal of registration.[16] Hence, although there is an association between pCR and event-free survival in specific cancer subtypes, a substantial improvement in pCR may not necessarily indicate a substantial improvement in

survival. Long-term follow results are, therefore, still required to make final recommendations.

For patients who do not achieve pCR, systems for pathologic tumour staging have been developed to characterize residual tumours, such as the Residual Cancer Burden (RCB) system and the ypT and ypN stage of the current American Joint Committee on Cancer (AJCC)/ International Union Against Cancer (UICC) staging system.[17,18] Campbell *et al.* showed that the RCB and the AJCC/UICC staging systems identify patients who are at highest risk for early recurrence, and combining pathologic staging with HR/HER2 subtyping further stratifies patients' risk.[19] It is known that patients with residual invasive disease after treatment with neoadjuvant anthracycline and taxane based chemotherapy have a 20 to 30% risk of relapse within 5 years and approximately half of the patients with triple-negative disease.[11] At this moment there are still controversies on how the information of residual invasive disease will be used for further treatment planning. Considering that residual disease may reflect resistance to the used neoadjuvant chemotherapy, it would make sense to use a non-cross-resistant postoperative systemic chemotherapy schedule. Recently, the CREATE-X trial showed that capecitabine was effective as adjuvant chemotherapy in patients with residual disease after neoadjuvant chemotherapy, with prolonged disease-free and overall survival.[20] This was especially seen in patients with triple negative disease with a 5-year disease-free survival of 69.8% in the capecitabine group versus 56.1% in the control group (HR 0.58; 95% CI 0.39-0.87) and a 5-year overall survival rate of 78.8% versus 70.3% (HR 0.52; 95% CI 0.30-0.90).[20] These results were in line with the results of a recent meta-analysis showing an improvement in disease-free and overall survival with the addition of capecitabine to standard chemotherapy in patients with triple negative disease.[21] Also some other trials are recruiting patients who did not achieved pCR after neoadjuvant chemotherapy for assessing the efficacy of additional non-cross resistant, adjuvant treatment. As an example the Olympia trial, assessing the efficacy (and safety) of olaparib as adjuvant treatment up to 12 months versus placebo as adjuvant treatment in patients with germline BRCA1/2 mutations and high-risk HER2-negative primary breast cancer after chemotherapy (NCT02032823).

We thus recognize that greater understanding of pathologic endpoints for neoadjuvant trials are necessary to design and interpret future clinical trials. Use of uniform staging definitions allows cancer outcomes to be placed into a proper context. Definitions of pCR varied, however, across trials. Absence of residual invasive disease after neoadjuvant chemotherapy in the breast, lymph nodes, or both, and including or excluding the *in situ* component was used. Nowadays the absence of the invasive component in both breast and lymph nodes (ypT0 ypN0 or ypT0/is ypN0; irrespective the *in-situ* component) is primarily used. But, although we agree that including nodal response to assess pCR rate is very important (as it has recently been shown to be extremely relevant for prognostication), we stress that patients with at

diagnosis clinical node-negative disease should be excluded when assessing nodal pCR rate. Most neoadjuvant trials have, however, included these cN0-patients, resulting in a high nodal pCR rate, whereas in most of these patients pN0 would already have been diagnosed, also if no neoadjuvant treatment was given. Therefore, in our study we calculated pCR rates of the lymph nodes as a secondary endpoint only in patients with clinical positive lymph nodes (preferably with cytological proof) at start of chemotherapy that were not removed by the sentinel node procedure.

Not only information on the pathologic response, but also reliable information on the clinical response during treatment is important for treatment decisions. In case of clinical tumour progression while on chemotherapy, treatment must be changed to immediate surgery or to another systemic treatment option. Clinical monitoring also identifies the size of residual disease to plan the most appropriate surgical approach. For the reasons mentioned above it is important that the clinical response predicts the pathological response accurately. We compared the clinical tumour size as assessed by magnetic resonance imaging (MRI) and ultrasound (US) post neoadjuvant chemotherapy with the postoperative pathologic tumour size. MRI and US showed comparable (moderate) agreement with post chemotherapy pathological tumour size with a low predictive value especially in ER-positive tumours. These data are relevant because with comparable agreement, ultrasound is the most available and cost-effective approach compared to MRI.
Recently, Lindenberg *et al.* explored differences in imaging performance with MRI and ^{18}FDG-PET/CT before and during neoadjuvant chemotherapy.[22] They concluded that there was a lack of evidence on the preferred imaging techniques per breast cancer subtype. New imaging approaches or combinations of existing imaging modalities (integrated PET-MRI) and use of fluorodeoxyglucose, ^{18}F-fluoroestradiol or ^{89}ZR-trastuzumab for response monitoring are currently being tested. More research is needed for optimal response monitoring per breast cancer subtype, possibly combined with biomarkers to predict the pathological response.

In patients with breast cancer, staging of the regional lymph nodes has traditionally been an integral part of disease management, as nodal status is considered an important prognostic factor. Axillary dissection provides excellent regional control in patients with nodal involvement, but is associated with more and often persistent side-effects, particularly lymph edema and restriction in shoulder mobility compared to sentinel lymph node biopsy.[23] In patients with clinical node negative disease a negative sentinel node can identify the patients without residual axillary disease to spare axillary lymph node dissection. The sentinel node can be performed pre-or post-neoadjuvant chemotherapy. Because neoadjuvant chemotherapy has shown to eradicate nodal disease in 20% to 40% of patients, performing a sentinel node procedure post neoadjuvant chemotherapy,[24,25] might further de-escalate treatment. In 2009 we provided an overview of the literature to determine the accuracy of a

sentinel node biopsy after neoadjuvant chemotherapy in clinically node negative and node positive patients. We calculated a pooled SN identification rate and false-negative rate of 90.9% and 10.5%, respectively. However, these rates were largely based on early SN studies. The reported pooled false-negative rate of 10.5% in this review is substantially higher than generally accepted without neoadjuvant chemotherapy and therefore we did not recommend the sentinel node biopsy following neoadjuvant chemotherapy as the standard of care. We recommended further research with subgroup analyses using variables reported to be associated with decreased sentinel node accuracy, including clinical nodal status, primary tumour size, body mass index and response to chemotherapy, in order to clearly define its value in subgroups of breast cancer patients.

More recent studies showed that a sentinel lymph node biopsy after neoadjuvant chemotherapy in clinically node negative patients had an accuracy similar to upfront sentinel lymph node biopsy.[26] The prospective European multi-centre GANEA study assessed the feasibility of sentinel lymph node biopsy after neoadjuvant chemotherapy in both clinical node positive and node negative patients.[27] Among 130 patients with cN0 disease, the sentinel lymph node identification rate was 95% with a false negative rate of 9% and a better detection rate compared to the 82% identification rate seen among patients with clinical node positive disease (P=.008).[27] We studied the impact of timing of the sentinel node procedure pre- versus post neoadjuvant chemotherapy in a selection of patients included in the INTENS trial and in the Dutch NEO-ZOTAC trial.[28] In our side-study, we concluded that in breast cancer patients with cN0 disease the sentinel node procedure performed post-neoadjuvant chemotherapy seemed to lead to nodal down staging (58% versus 51%), although this difference was not statistically significant. The absence of regional recurrences after a median follow-up of six years in those who did not undergo completion axillary lymph node dissection underscores the conclusion that performing the sentinel node procedure post-chemotherapy is safe in patients with clinically node-negative breast cancer at initial diagnosis. Based on our and other studies, we conclude that in breast cancer patients with clinically node-negative disease the sentinel node procedure is preferentially postponed till after the end of neoadjuvant chemotherapy to take maximum benefit of its effect on nodal down staging of subclinical disease.

In patients with clinically node-positive disease previous to neoadjuvant chemotherapy, three recently published prospective studies, ACOSOG Z1071, SENTINA and SN FNAC showed a false negative rate of approximately 13%, with the false negative rate directly related to the applied technique.[29-32] The reliability of the sentinel node procedure was enhanced by using both radiolabeled colloid and methylene blue (false-negative rate 10.8%) and by removing a higher number of nodes (false negative rate 9.1%).[29-32] Nevertheless, techniques for more reliable identification of possibly involved nodes need yet further testing. To be able to further

de-escalate axillary treatment we should think of new ways to identify the nodal status of patients and to use new techniques or combination of existing techniques.[29-32] An alternative method is the use of a marker, e.g. radioactive iodine seed, selectively placed into the clinically most suspected positive-node.[33] Another strategy is the combination of the sentinel node procedure and a marked node procedure (e.g. radioactive iodine seed, clip placement or preoperative tattooing of the clinical positive-node) in patients with initially clinical node-positive disease.[33-35] Boughey *et al.* reported a false negative rate of 6.8% (95% CI 1.9-16.5%) in patients with clip placement of the initially clinical node positive disease and a sentinel node procedure after neoadjuvant chemotherapy with removal of at least 2 sentinel nodes followed by axillary dissection.[35] Caudle *et al*. reported in a prospective single-institution study with 208 breast cancer patients a false negative rate of 10.1% (95% CI 4.2-19.8) when only a sentinel node procedure was done after neoadjuvant chemotherapy, evaluation of the clipped node resulted in a false negative rate of 4.2% (95% CI 1.4-9.5) and the combination of sentinel node and a clipped node reduced the false negative rate to 1.4% (95% CI 0.03-7.3).[36] Whether the combination procedures are technically feasible in daily practice and whether the negative predictive failure indeed improves by the combined technique needs to be confirmed in a multicentre setting before broadly implementing this technique in daily practice. Currently, in the Netherlands the RISAS study, combining radioactive iodine seed localization in the axilla in axillary node positive breast cancer with a sentinel node procedure, has been launched to address this further (NCT02800317). In the studies reported so far, long-term outcome of patients who converted from clinical-positive to sentinel node-negative without completion axillary lymph node dissection is not available. Residual disease is per definition resistant for the neoadjuvant chemotherapy given earlier and there might be an increased risk for regional recurrence if positive non-sentinel nodes are left behind.

In the adjuvant setting the ACOSOG Z0011 trial (2011) randomised patients with clinical T1–T2 breast cancer and a positive sentinel node who were treated with lumpectomy, adjuvant systemic therapy, and tangential field whole-breast radiotherapy to axillary surgery or a wait and see policy.[37] After a median follow up of 6.3 years axillary lymph node dissection did not significantly affect locoregional control or survival, although it was debated afterwards whether in some patients who did not undergo axillary surgery, axillary radiotherapy had yet been offered. The phase 3 AMAROS trial showed that axillary radiotherapy and axillary lymph node dissection both provided excellent and comparable locoregional control in patients with T1–2 primary breast cancer and no palpable lymphadenopathy who were found to have a positive sentinel lymph node.[38] The patients participating in the ACOSOG Z0011 or AMAROS study were mostly also treated with *adjuvant* systemic treatment. In the *neoadjuvant* setting, however, for patients with node positive disease the optimal treatment regarding axillary therapy still remains unclear. The randomised

phase III ALLIANCE A 011202 (NCT01901094) study will evaluate whether nodal and axillary radiation therapy is as effective as axillary lymph node dissection combined with nodal radiation in patients treated with neoadjuvant chemotherapy. We need to wait for the results but more research needs to be conducted regarding less invasive axillary treatment in patients with favourable response to neoadjuvant chemotherapy.

In conclusion, we have shown that sequential anthracycline and taxane based chemotherapy has a superior outcome and compared to other trials sequential treatment was never inferior to concurrent delivered anthracycline and taxane based triplet chemotherapy with a lower cumulative dose of anthracyclines. Further research is needed to avoid overtreatment in patients with pCR in breast and axilla (de-escalation) as well as on improved therapy options (escalation) for patients with non-pCR in breast and axilla after standard neoadjuvant therapy. New imaging approaches or combinations of existing imaging modalities for response monitoring could be considered to de-escalate or escalate treatment regarding adjuvant systemic therapy or treatment of the axilla. It is important to consider that residual disease in both breast and axilla after neoadjuvant chemotherapy (ypTN-status) may reflect resistance to the used neoadjuvant chemotherapy and requires a different interpretation than the same tumour staging (pTN-status) in the adjuvant setting.

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Valorisation

Valorisation

This thesis addresses several aspects of neoadjuvant chemotherapy in breast cancer patients in relation to efficacy, response assessment and axillary strategy. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide. The incidence of invasive breast cancer in the Netherlands increased the last decades to 14,551 patients in 2015. Due to its high incidence, breast cancer has an important societal and economic impact in the Netherlands, with annual incremental costs.
Over the past decades we have seen a significant decrease of mortality of early breast cancer due to early detection, multidisciplinary approach and improved options for systemic treatment, i.e., chemotherapy, HER2 targeted therapy and endocrine therapy. With the addition of taxanes to anthracyclines and cyclophosphamide in recent years, overall survival has clearly further improved. Quality of life of survivors is affected by the morbidity caused by this treatment. Recommendations for the use of systemic therapy are based on the individual patient's risk and the balance between absolute benefit and toxicity. Overall, chemotherapy regimens based on anthracyclines and taxanes reduce breast cancer mortality by about one-third.

This thesis is mainly based on the data of the Dutch phase III INTENS study, comparing sequential use of docetaxel after anthracyclines and cyclophosphamide (AC-T) and the upfront use of the triple combination of docetaxel with anthracyclines and cyclophosphamide (TAC). Both regimens showed to be equally effective in achieving a pathologic complete response (pCR). After a median follow-up of six years, however, sequentially delivered AC-T showed a superior disease-free and overall survival for all patient subgroups compared to concurrent TAC chemotherapy. Not only improved outcome, moreover, the lower cumulative anthracycline dose with AC-T is attractive because of the lower risk of cardiotoxicity and risk of leukemia. Also, the reduced use of G-CSF during AC-T therapy, for only four cycles, in comparison with the use of G-CSF during all 6 cycles of TAC chemotherapy, can be considered as an advantage for sequentially delivered chemotherapy.

Clinical monitoring identifies the size of residual disease to plan the most appropriate surgical approach. It is important that the clinical response predicts the pathological response accurately. We compared the clinical tumour size as assessed by magnetic resonance imaging (MRI) and ultrasound (US) post neoadjuvant chemotherapy with the postoperative pathologic tumour size. MRI and US showed comparable (moderate) agreement with post chemotherapy pathological tumour size with a low predictive value especially in ER-positive tumours. These data are relevant because with comparable agreement, ultrasound is the most available and cost-effective approach compared to MRI.

Surgery for breast cancer has become less radical than it was several decades ago. For patients with earlier stages of breast cancer, down staging of the primary tumour may facilitate breast conserving therapy and bears the opportunity of down staging the axilla obviating the need of axillary treatment in some patients. For the axilla, the sentinel node biopsy has replaced the axillary lymph node dissection as a staging procedure. Axillary dissection provides excellent regional control in patients with nodal involvement, but is associated with more and often persistent side-effects, particularly lymph edema and restriction in shoulder mobility compared to sentinel lymph node biopsy. In patients with clinical node negative disease, a negative sentinel node can identify the patients without residual axillary disease to spare axillary lymph node dissection. The sentinel node can be performed pre-or post neoadjuvant chemotherapy. Because neoadjuvant chemotherapy has shown to eradicate nodal disease in 20% to 40% of patients, performing a sentinel node procedure post neoadjuvant chemotherapy might further de-escalate treatment. Based on our and other studies, we conclude that in breast cancer patients with clinically node-negative disease the sentinel node procedure is preferentially postponed till after the end of neoadjuvant chemotherapy to take maximum benefit of its effect on nodal down staging of subclinical disease.

In addition to the academic community, especially the clinicians who take part in multidisciplinary breast cancer treatment for breast cancer patients, the results of this thesis are relevant for newly diagnosed breast cancer patients with indication for systemic treatment because of the improved treatment option with least risk of long term toxicities. Also, the contents of this thesis are of interest to expert panels, which are responsible for the development of the national and international guidelines in breast cancer treatment.

Further research is needed to improve treatment options for patients with breast cancer with enhanced attention to avoid overtreatment in patients with pCR in breast and axilla (de-escalation) as well as on improved therapy options (escalation) for patients with non-pCR in breast and axilla after standard neoadjuvant therapy. More research is needed for optimal response monitoring, new imaging approaches or combinations of existing imaging modalities for response monitoring could be considered, possibly combined with biomarkers to predict the pathological response.

In conclusion, this thesis provides essential data of several aspects of neoadjuvant chemotherapy in breast cancer patients in relation to efficacy, response assessment and axillary strategy. These data will contribute to further improvement of breast cancer treatment and outcome.

List of publications

List of publications

M.J.B. Aarts, **B.E.P.J. Vriens**, M. de Boer, F.P.J. Peters, C.M.P.W. Mandigers, M.W. Dercksen, J.M.L. Stouthard, J. Tol, L.J.C. van Warmerdam, A.J. van de Wouw, E.M.G. Jacobs, C.C.D. van der Rijt, T.J. Smilde, A.W.G. van der Velden, P.G.M. Peer, V.C.G. Tjan-Heijnen. Neutrophil recovery in breast cancer patients receiving docetaxel-containing chemotherapy with and without G-CSF prophylaxis. Oncology 2017; Aug 22: Epub ahead of print.

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Dankwoord

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Curriculum vitae

Curriculum vitae

Birgitte Elisabeth Petronella Johanna (Birgit) Vriens werd op 23 september 1975 geboren in Boxmeer en groeide op in Moergestel. Na het afronden van het VWO op het St. Odulphuslyceum te Tilburg studeerde zij Gezondheidswetenschappen (afstudeerrichting Biologische Gezondheidskunde) aan de Universiteit van Maastricht en behaalde haar doctoraal examen in 1999.
In 1998 begon zij aan de studie geneeskunde aan de Universiteit van Maastricht en behaalde het basisarts examen in 2003. In datzelfde jaar begon zij met de opleiding tot internist in het Catharina Ziekenhuis in Eindhoven (opleider dr. B. Bravenboer). In 2007 zette zij haar opleiding voort in het academisch ziekenhuis Maastricht, thans Maastricht Universitair Medisch Centrum (opleider Prof. Dr. C.D.A. Stehouwer) en startte zij met het aandachtsgebied medische oncologie (opleider Prof. V.C.G. Tjan –Heijnen). Tijdens de opleiding tot medisch oncoloog begon zij met onderzoek in kader van dit proefschrift. In 2009 volgde de registratie als internist-oncoloog. Hierna was zij als staflid internist-oncoloog werkzaam in het Maastricht Universitair Medisch Centrum. Vanaf 2012 is zij werkzaam als internist-oncoloog bij de vakgroep interne geneeskunde/MDL in het Catharina Ziekenhuis te Eindhoven.
Zij is lid van de commissie Beoordeling van Oncologische middelen (BOM) van de Nederlandse Vereniging voor Medische Oncologie (NVMO) en lid van de werkgroep revisie landelijke richtlijn mammacarcinoom namens de Nederlandse Internisten Vereniging/NVMO.